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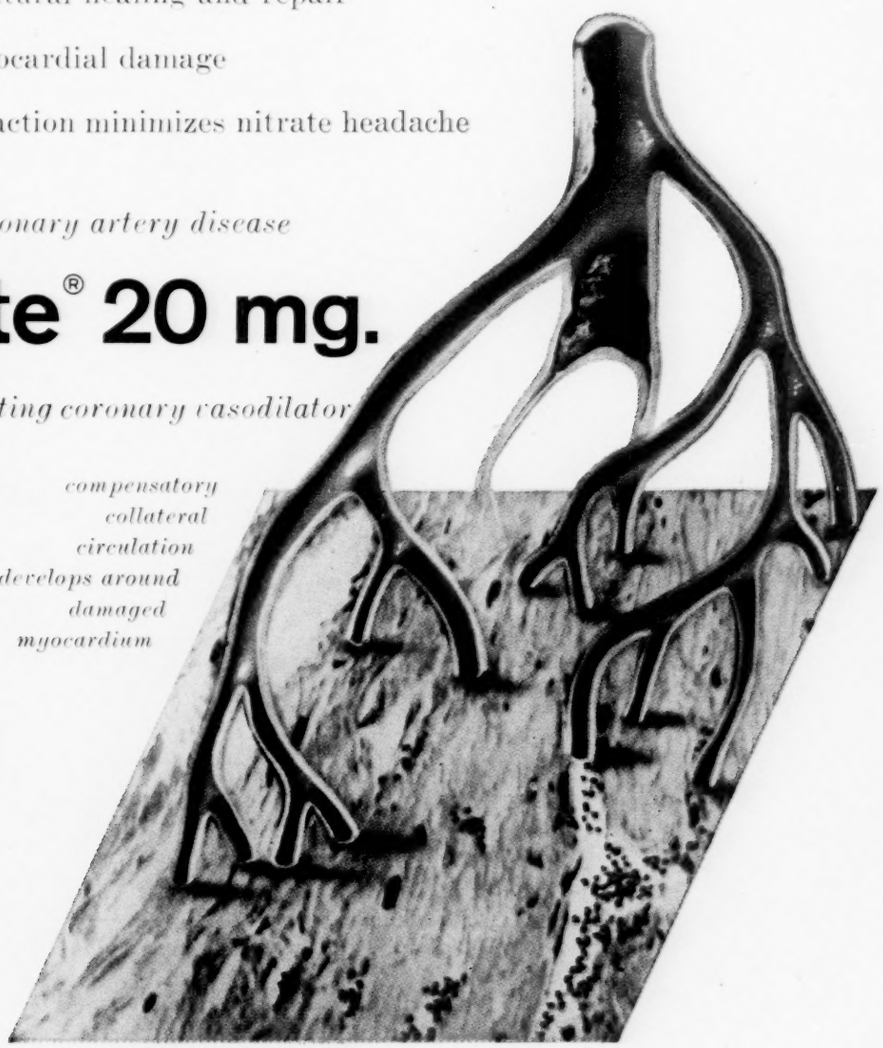
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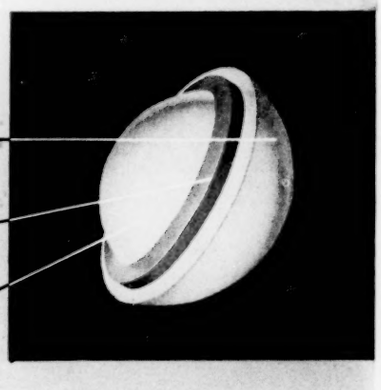
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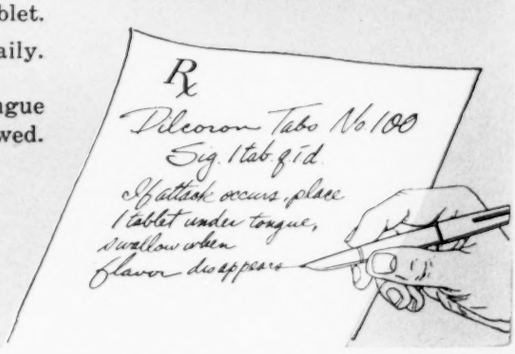


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The American Journal of Medicine

Vol. XXIX JULY 1960 No. 1

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Hereditary Persistence of Fetal Hemoglobin. A Family Study
Emery C. Herman, Jr. and C. Lockard Conley 9

This paper points up certain inadequacies of filter paper electrophoresis as a means of differentiating sickle cell anemia (S-S disease) from other sickling disorders, and calls attention in this connection particularly to the association of hemoglobin S with persistent fetal hemoglobin. The clinical symptomatology of this combination of hemoglobinopathies is much less pronounced than in sickle cell anemia, which is what attracted the notice of the investigators. The hereditary nature of persistence of fetal hemoglobin in this context, and the implications thereof, are discussed informatively.

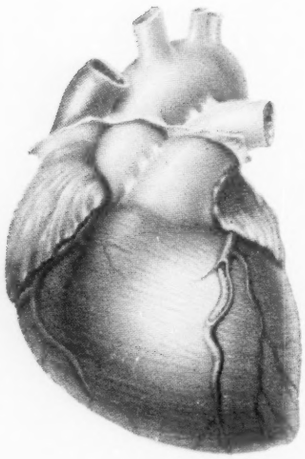
Hemoglobin Metabolism in Thalassemia. In Vivo Studies
Moises Grinstein, Robin M. Bannerman, John D. Vavra and Carl V. Moore 18

By tracing the incorporation of glycine-2-C^{14} into heme, a defect in hemoglobin synthesis could be demonstrated in patients with thalassemia major. This, then, is another important factor in the anemia of that disorder (in addition to the long known role of hemolysis and relative failure of the bone marrow) and indicative of a genetically derived defect in the enzyme complement of the erythroblast. A possible clue to the nature of this metabolic aberration was obtained in the excessive early labeling of stercobilin noted, suggesting diversion of glycine from the pathways of heme biosynthesis into alternate pathways as a consequence of the enzyme lack.

Familial Hypogammaglobulinemia, Splenomegaly and Leukopenia. With a Review of the Etiological Factors of the Hypogammaglobulinemias
Daniel S. Kushner, Alvin Dubin, William P. Donlon and David Bronsky 33

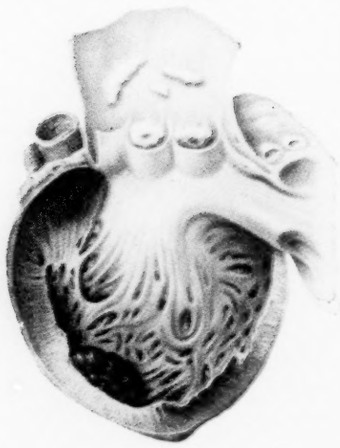
An interesting variant is added to the recently described spectrum of the hypogammaglobulinemias viz. hypogammaglobulinemia associated with leukopenia and splenomegaly appearing as a familial disturbance and perhaps transmitted as an autosomal dominant gene. A review of the known etiological factors of the hypogammaglobulinemias is presented.

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1. Ellis, L. B. *et al.*: Circulation *17*:945, May 1958. **2.** Friedlander, H. S.: Am. J. Cardiol. *1*:395, Mar. 1958. **3.** Riseman, J. E. F.: New England J. Med. *261*:1017, Nov. 12, 1959. **4.** Russek, H. I. *et al.*: Circulation *12*:169, Aug. 1955. **5.** Russek, H. I.: Am. J. Cardiol. *3*:547, April 1959. **6.** Tortora, A. R.: Delaware M. J. *30*:298, Oct. 1958. **7.** Waldman, S. and Pelner, L.: Am. Pract. & Digest Treat. *8*:1075, July 1957.

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VOLUME TWENTY-NINE NUMBER ONE

Serum Alkaline Phosphatase Activity in Hepatitis of Infectious Mononucleosis
JEREMIAH A. BARONDESS AND HENRY ERLE 43

The present study provides additional data on the increase in serum alkaline phosphatase, often associated with little or no jaundice, in patients with infectious mononucleosis and liver involvement. In ten of seventy-three cases, the phosphatase exceeded 20 Bodansky units; in most of the others the values exceeded the upper limits of normal. The significance of these findings is discussed.

The Cholestatic Form of Viral Hepatitis. Experiences with Viral Hepatitis at Brooke Army Hospital During the Years 1951 to 1953
I. N. DUBIN, B. H. SULLIVAN, JR., P. C. LEGOLVAN AND L. C. MURPHY 55

The senior author, an old hand at diseases of the liver, encountered a minor epidemic of infectious viral hepatitis which, liver biopsies disclosed, exhibited an unusually high proportion of patients with uncommonly pronounced cholestasis. A detailed analysis of the clinical, laboratory and pathologic findings follows, in context with a sophisticated discussion of the general problem of the cholestatic form of viral hepatitis—a still confused and controversial subject. It is becoming clear that there is more or less bile stasis in virtually every liver deeply affected by the hepatitis virus, and one might well argue whether the cut-off point arbitrarily selected by the authors is justified. Nevertheless, the fact is that some livers show much more cholestasis than others, and the possible reasons therefor are knowingly discussed. One possibility lies in variant strains of virus.

Primary Mediastinal Echinococcosis . . . . J. RAKOWER AND H. MILWIDSKY 73

While the problem discussed here is of concern chiefly in endemic centers of echinococcosis, cases of hydatid disease may turn up sporadically almost anywhere, and should enter into the differential diagnosis. A primary mediastinal location is distinctly uncommon, difficult of diagnosis, and, when symptomatic, varied in its clinical manifestations. When broken down, as in this scholarly study, into posterior, middle and anterior mediastinal location, however, it is possible to derive the clinical signs and symptoms in a rational manner.

Course and Prognosis of Sarcoidosis . . MAURICE SONES AND HAROLD L. ISRAEL 84

This analysis of a large experience with proved sarcoidosis (211 cases) in Philadelphia over a protracted period of observation (mean, approximately six years) brings out several interesting and some debatable points. There was a moderate but significant mortality (9.5 per cent), particularly in Negro patients, even in this series conspicuous for predominantly early cases. Recurrence after complete clearing was rare. Tuberculosis was a relatively uncommon accompaniment, and not more frequent in corticosteroid-treated patients. The incidence of erythema nodosum in this series was unusually low. Cutaneous involvement correlated more closely than any other manifestation with progressive and fatal disease.

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A urine culture is absolutely essential in the diabetic suspected of having a urinary tract infection since such infection is not always accompanied by pyuria. It is also essential to keep the urine free from sugar—as shown by frequent urine-sugar tests—for successful therapy.

Source: Harrison, T. R., *et al.:* Principles of Internal Medicine, ed. 3, New York, McGraw-Hill Book Co., 1958, p. 620.

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An "Epidemic" of Chemical Meningitis
WINFREY W. GOLDMAN, JR. AND JAY P. SANFORD 94

The authors make a point worthy of consideration, namely, the occurrence of puzzling symptoms and signs of meningitis following spinal anesthesia or intrathecal injection of antibiotics, due to the inadvertent persistence of traces of phenolic disinfectants or detergents in the syringes employed. In the five illustrative cases cited this "chemical meningitis" fortunately was transient, with complete recovery, but more serious reactions may ensue.

Reviews

The Liver Cell. Some New Approaches to Its Study
ALEX B. NOVIKOFF AND EDWARD ESSNER 102

Electron microscopy, subcellular enzyme localization by histochemical technics, differential centrifugation and other refined methods of investigation have added a new dimension to exploration of cell structure and function. The results of such studies are here brought to bear upon the new concepts of the hepatic units of cellular morphology and physiology—for at this macromolecular level structure and function become one. The discussion includes a consideration of the cytoplasmic components (mitochondria, endoplasmic reticulum, lysosomes, etc.) and nucleus of the parenchymal cell, the nature of the bile canaliculi and of the Kupffer cells. Touched upon are such basic problems as the site and mechanisms of biosynthesis, the apparatus of transcellular transfer and the mechanics of phagocytosis.

Renal Medullary Necrosis
DAVID P. LAULER, GEORGE E. SCHREINER AND ARTHUR DAVID 132

It is becoming increasingly apparent that renal medullary necrosis is neither excessively rare nor impossible of diagnosis during life. A full account of the disorder is given in the present study which brings out a number of interesting features presented by the six cases described by the authors and 250 cases included in a comprehensive review of the literature. Most commonly encountered in diabetic subjects, and in association with obstructive uropathy in non-diabetic subjects, renal medullary necrosis clearly is an extreme form of pyelonephritis, its development all too frequently initiated or accelerated by iatrogenic factors. The success of management depends upon eradication of infection and other predisposing causes. Recovery or protracted course occurs more commonly than is generally appreciated, as is made clear, and diagnosis therefore is of more than academic interest.

Clinicopathologic Conference

Progressive Hepatic Failure 157

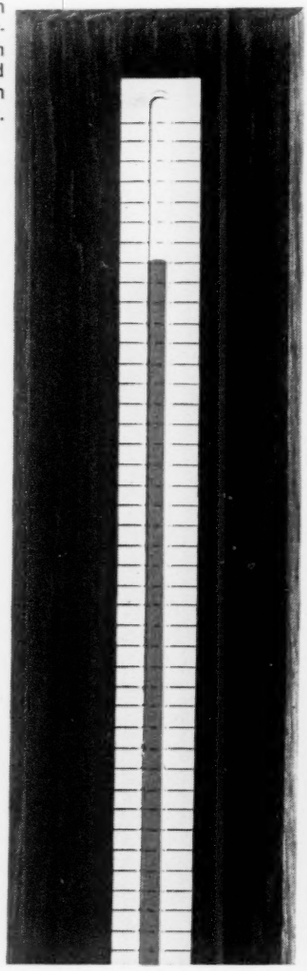
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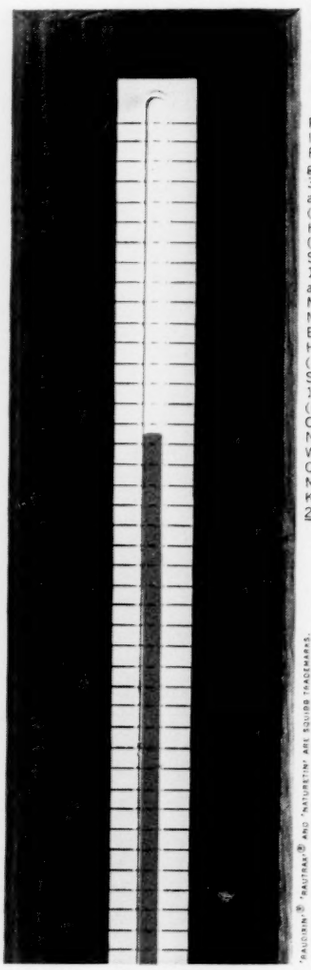
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Case Reports

Studies on Renal Enzymes in a Patient with Renal Tubular Acidosis
SUMNER J. YAFFE, JOHN M. CRAIG AND FRANCIS X. FELLERS 168

This study of a case of renal tubular acidosis brings out several interesting points, including direct demonstration of a sufficiency of carbonic anhydrase in kidney tissue procured by biopsy.

Disseminated Atheromatous Emboli
EDWIN R. FISHER, H. RICHARD HELLSTROM AND JACK D. MYERS 176

An interesting example of multiple atheromatous embolization, a condition which, as the authors state, doubtless occurs more frequently than recognized by clinician or pathologist.

Acute Gaseous Cholecystitis . . . . . F. CALVIN BIGLER 181

An interesting report.

Advertising Index on Pages 137 and 138

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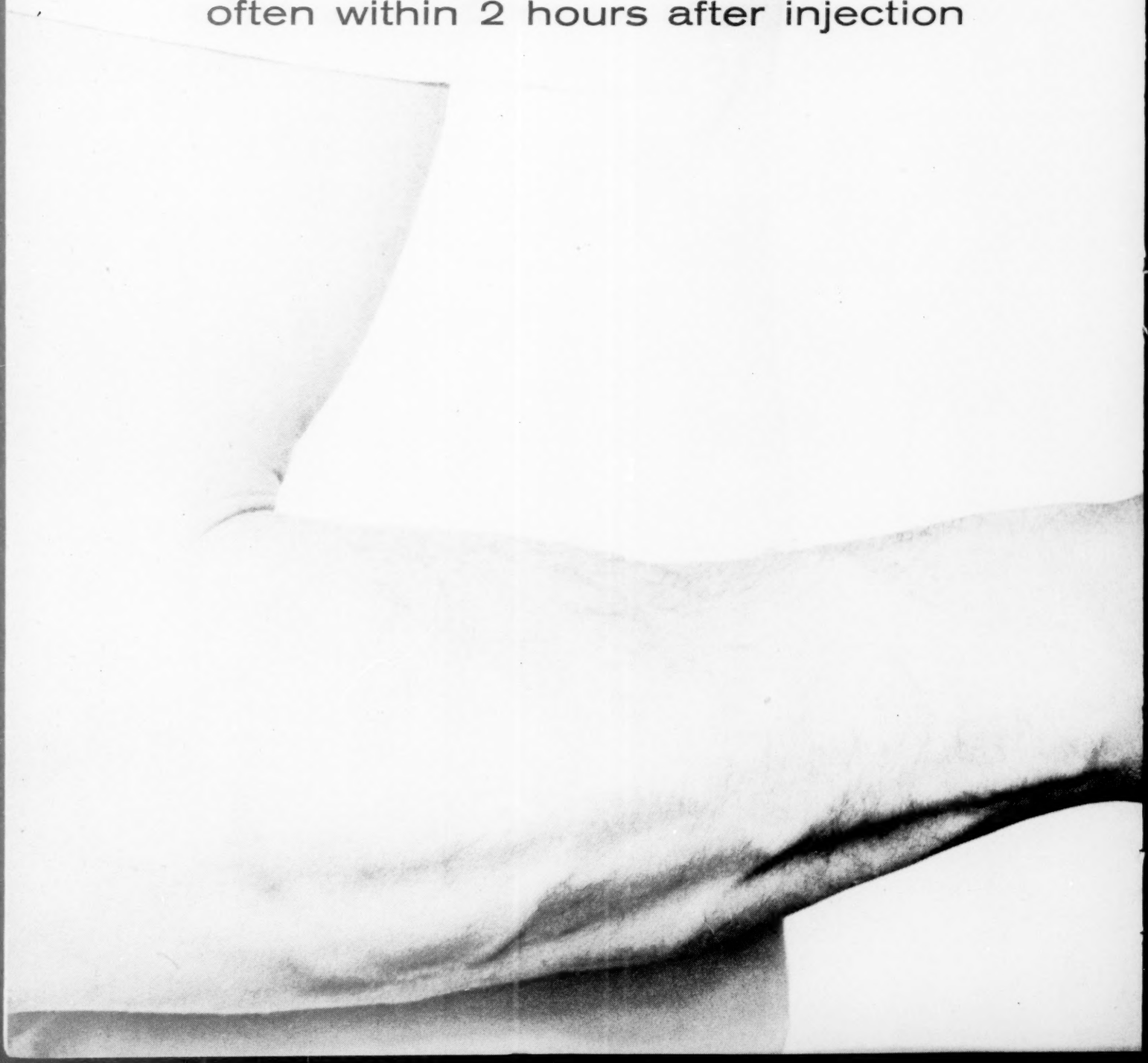
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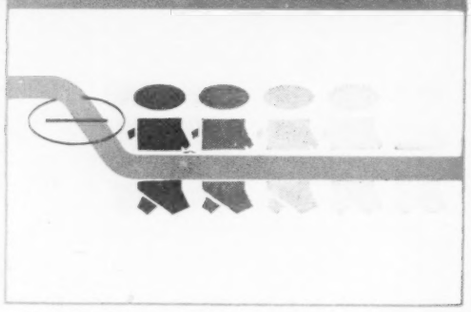
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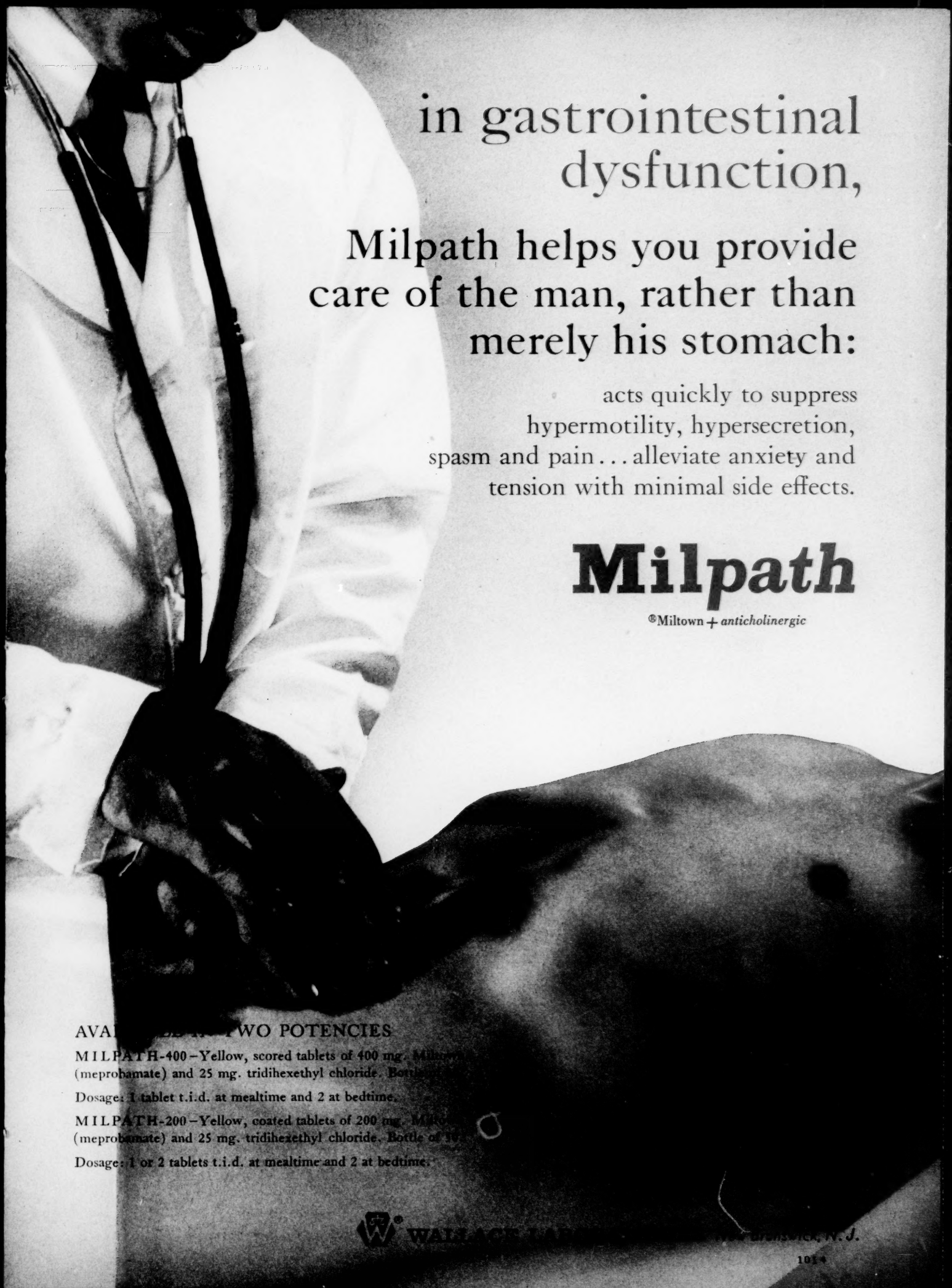
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MILPATH-400—Yellow, scored tablets of 400 mg. Milt[illegible] (meprobamate) and 25 mg. tridihexethyl chloride. Bot[illegible]

Dosage: 1 tablet t.i.d. at mealtime and 2 at bedtime.

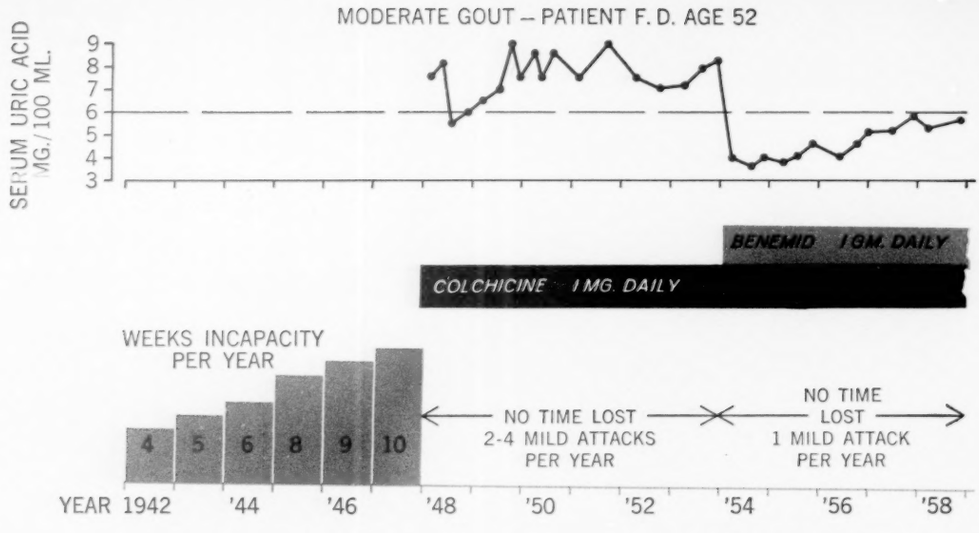
MILPATH-200—Yellow, coated tablets of 200 mg. M[illegible] (meprobamate) and 25 mg. tridihexethyl chloride. Bottle of [illegible]

Dosage: 1 or 2 tablets t.i.d. at mealtime and 2 at bedtime.

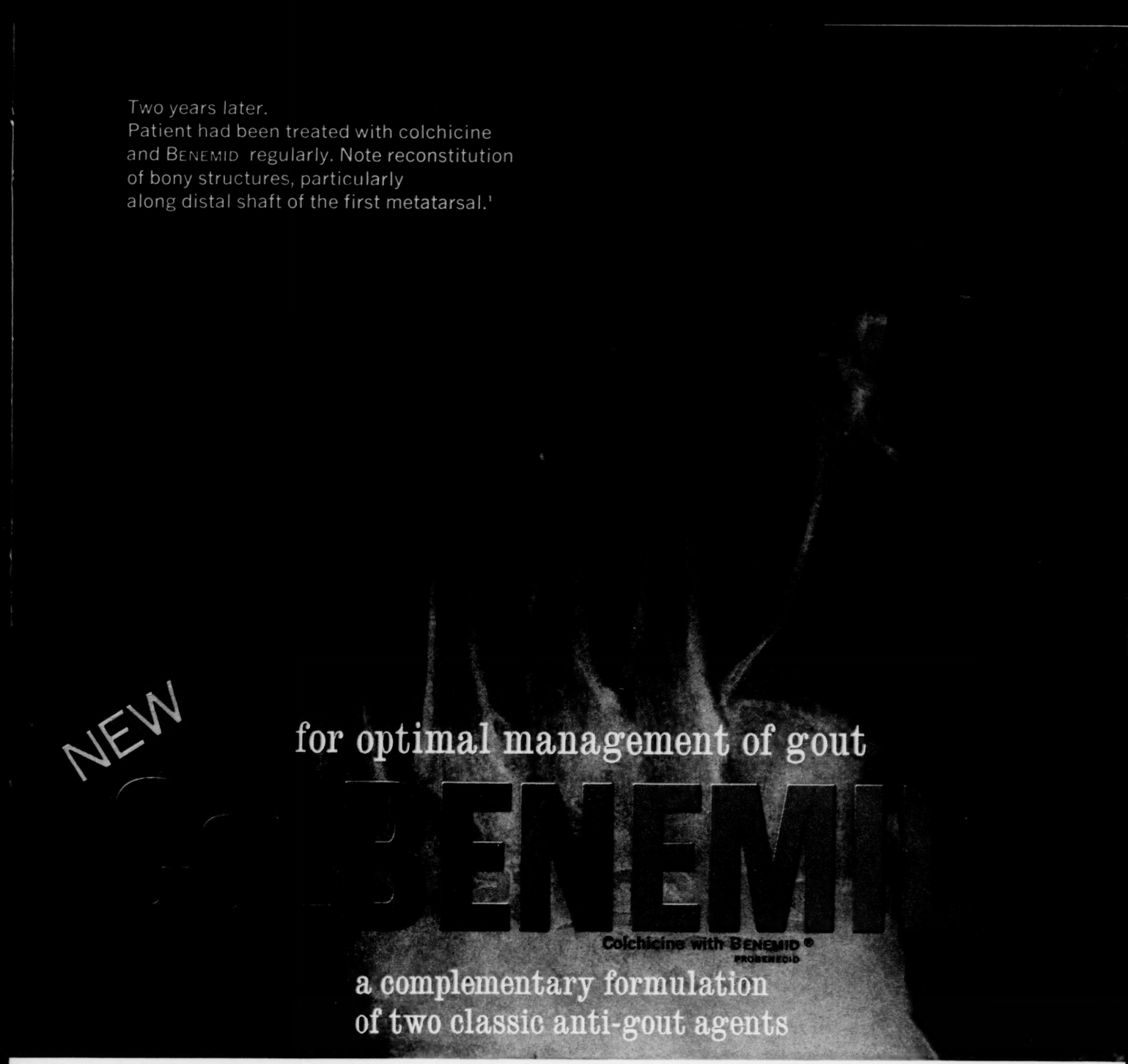
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1014

Before treatment.
Extensive gouty changes in base of proximal phalanx of great toe and in head and shaft of the first metatarsal.



Effect of colchicine and BENEMID on serum uric acid level and periods of incapacity.[2]



"Prophylactic management [of gout] embodies the use of the two agents just discussed, namely, colchicine and Benemid. Each one complements the other. Neither one by itself is as effective as a combination.... Since 1950, Benemid has been available and the greater the experience we have with the combination of colchicine and Benemid the greater the reliance we place upon these two drugs."[3]

Composition: Each tablet contains 0.5 mg. colchicine and 0.5 Gm. BENEMID probenecid.

Dosage between acute episodes: Mild, 1 tablet a day; moderate, 1 tablet twice daily; severe, 1 tablet three or more times daily.

Supply: Bottles of 100.

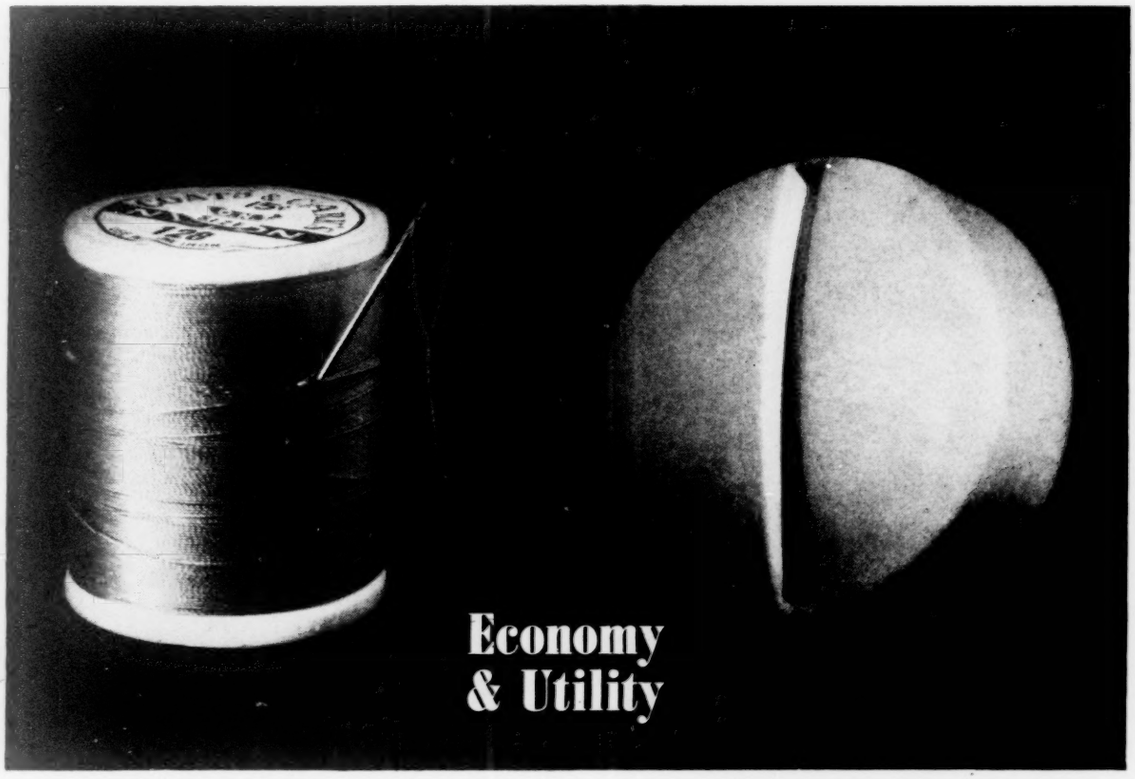
1. Talbott, J. H.: Gout, New York, Grune & Stratton, 1957, pp. 162, 163. 2. Talbott, J. H.: Gouty arthritis, Minn. Med. *42*:1044, Aug. 1959. 3. Talbott, J. H.: Recognition and treatment of gouty arthritis, Current Medical Digest *26*:57, Nov. 1959.

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Pentids

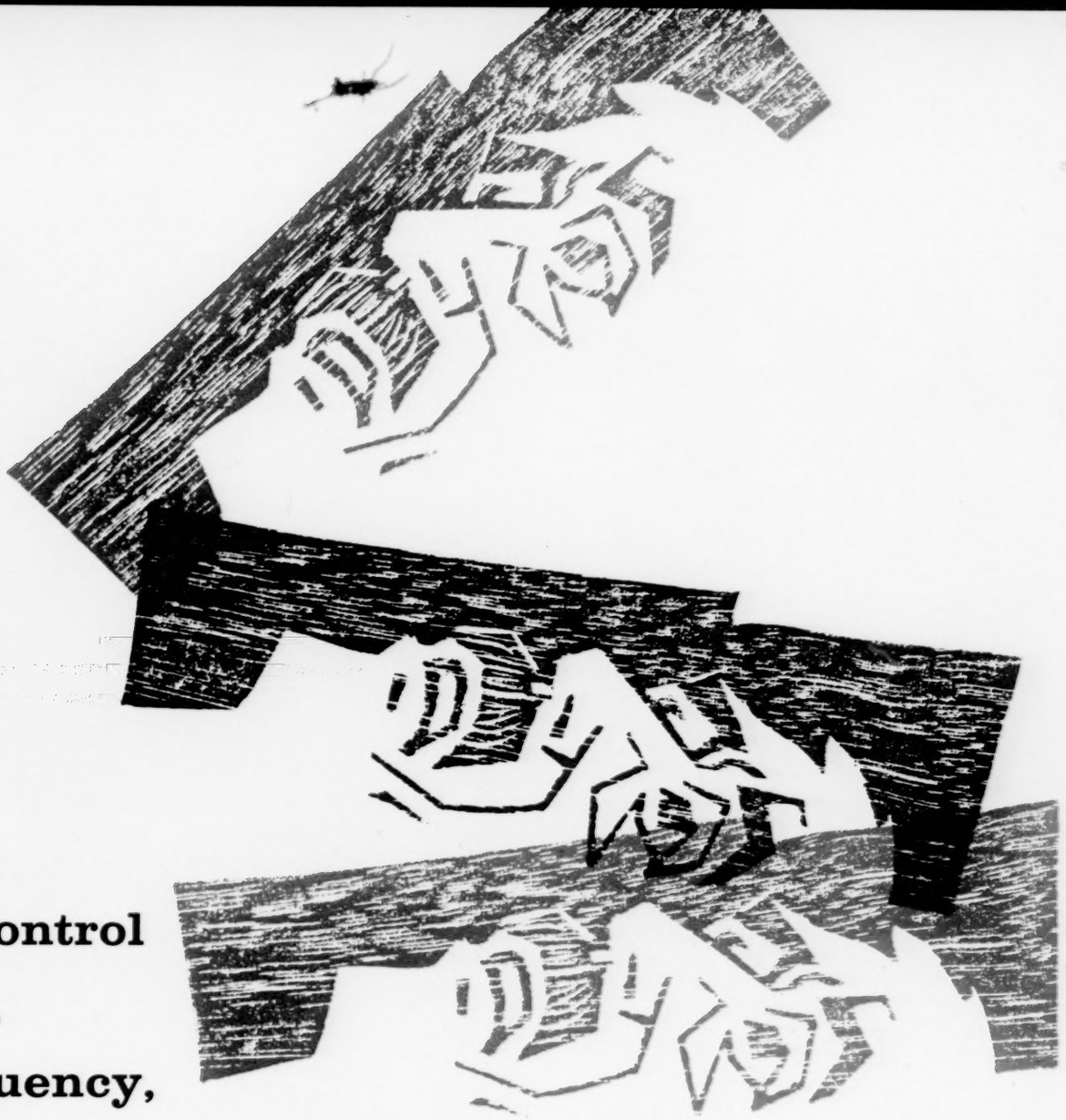
Squibb Penicillin G Potassium

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EQUANITRATE helps control pain and accompanying anxiety in angina pectoris. It reduces the number and severity of attacks, increases exercise tolerance, and lessens nitroglycerin dependence.

A recent double-blind study† comparing meprobamate, a placebo, PETN, and EQUANITRATE states: "The best results . . . in both clinical and electrocardiographic response, were observed with a combination of meprobamate and pentaerythritol tetranitrate [EQUANITRATE]. . . ."

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†Russek, H.I.: Am. J. Cardiol. *3*:547 (April) 1959.

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Supplied: **EQUANITRATE 10** (200 mg. meprobamate, 10 mg. pentaerythritol tetranitrate), white oval tablets, vials of 50. **EQUANITRATE 20** (200 mg. meprobamate, 20 mg. pentaerythritol tetranitrate), yellow oval tablets, vials of 50.



Photos used with patient's permission.

How new **Dianabol** rebuilt muscle tissue in this underweight, debilitated patient

Patient was weak and emaciated before Dianabol. R. C., age 51, weighed 160 pounds following surgery to close a perforated duodenal ulcer. His convalescence was slow and stormy, complicated by pneumonia of both lower lobes. Weak and washed out, he was considered a poor risk for further necessary surgery (cholecystectomy). Because a conventional low-fat diet and multiple-vitamin therapy failed to build up R. C. sufficiently, his physician prescribed Dianabol 5 mg. b.i.d.

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And because Dianabol is low in cost, it is particularly suitable for the aged or chronically ill patient who may require long-term anabolic therapy.

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... Initial dosage: 2 capsules 3 ... Maintenance dosage to be ... to response.

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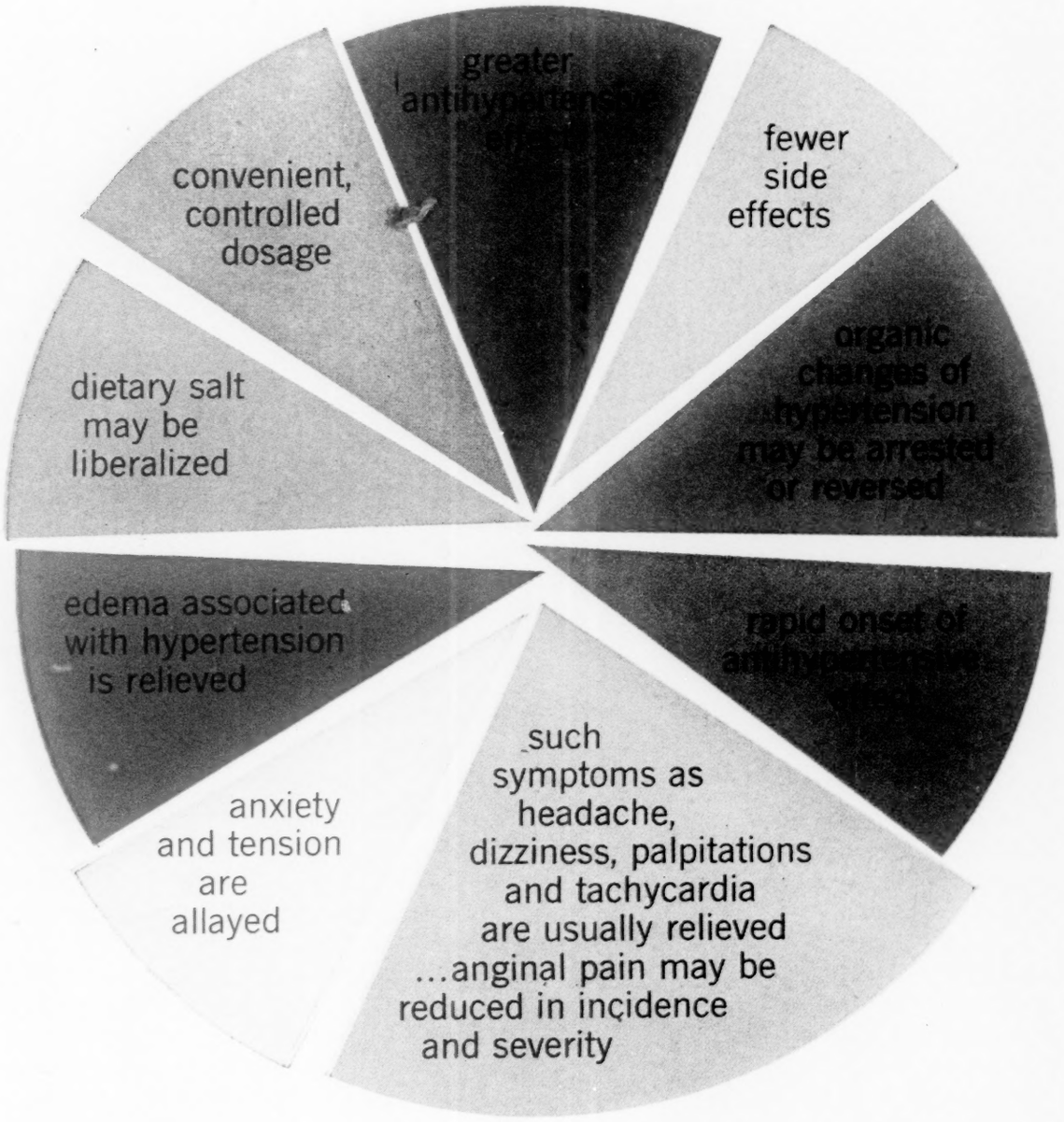
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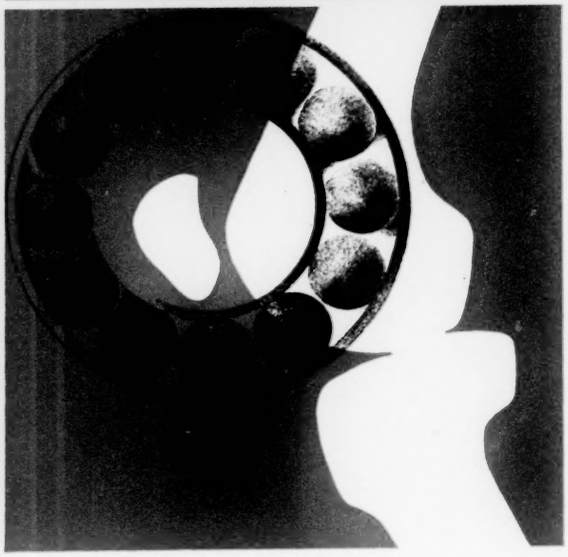
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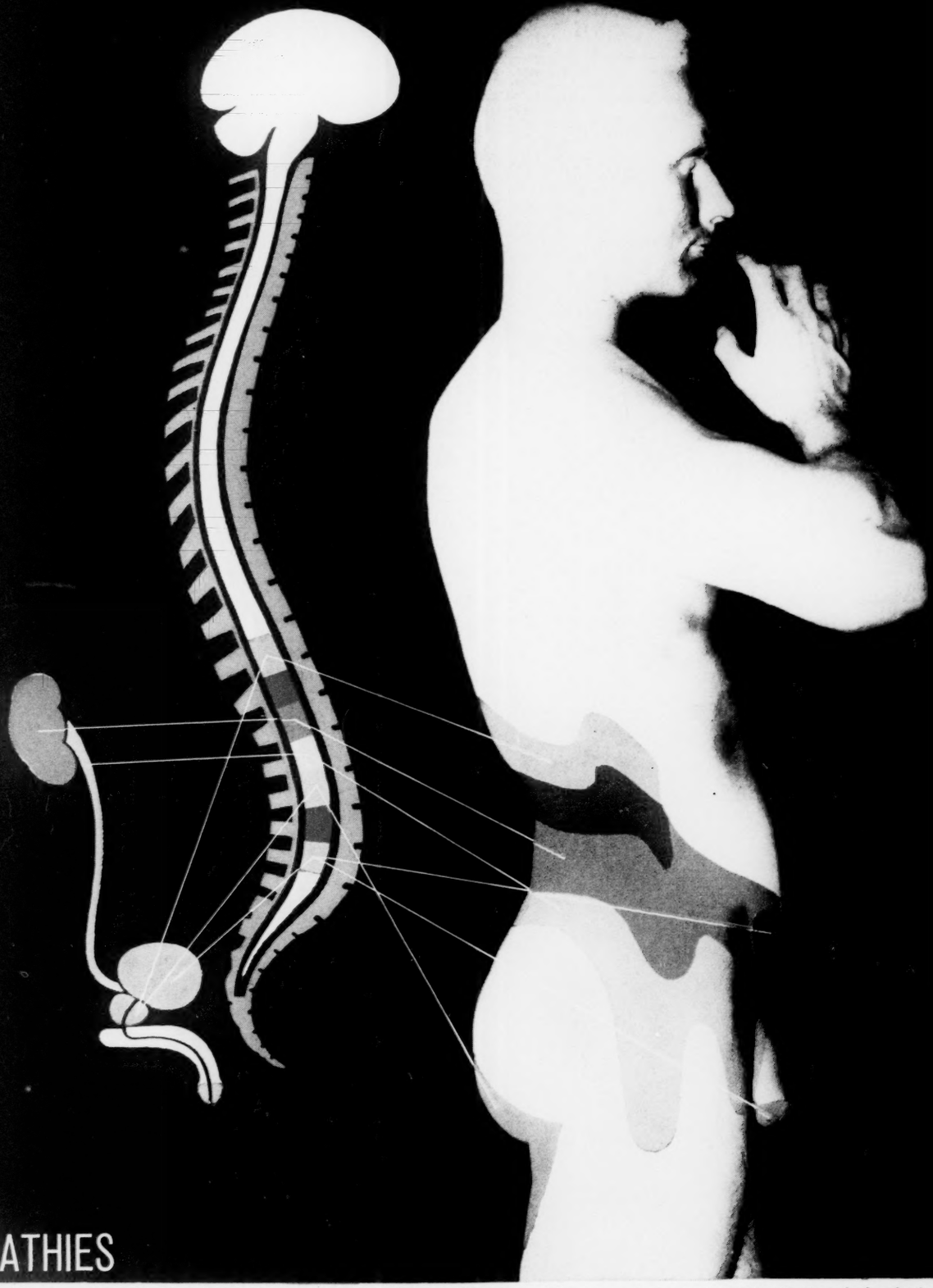


Naqua™
trichlormethiazide

lowest dosage, balanced
sodium and chloride output in

edema

S-485



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WHEN PAIN IS PART OF THE PICTURE

Diagram correlates sources of primary urinary pain with **areas of referred pain.**

Urinary tract pain, at the source or referred, is subject to the rapid analgesic action of the azo dye in Azo Gantrisin. Azo Gantrisin combines dramatic relief of symptoms with proven effective action against infections carried by either blood stream or urine.

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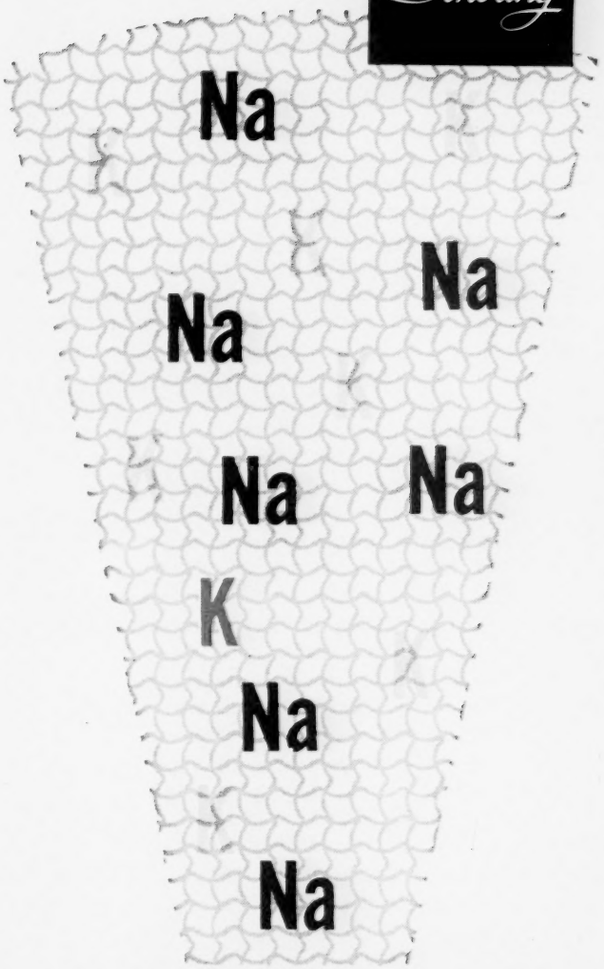
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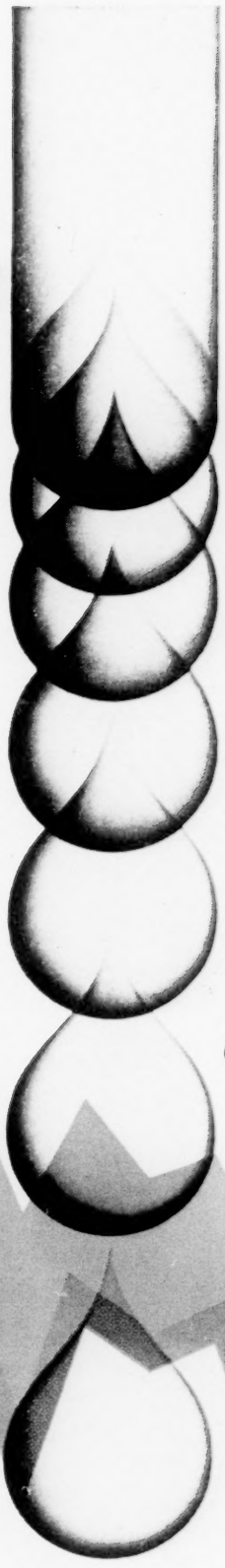
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brand of chlorthalidone

longest in action... smoothest in effect

in hypertension and edema



greater loss of sodium
lesser loss of potassium

A new antihypertensive-saluretic, Hygroton, now enables still more effective control of hypertension and edema.

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Because it is more prolonged in action than any other diuretic,[1] Hygroton affords a smoother, more evenly sustained response.

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Hygroton produces only minimal potassium loss . . . affords a better sodium-potassium ratio than other saluretics.[3]

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As a rule, with Hygroton, restriction of dietary salt is unnecessary.

more convenience and economy
For maintenance therapy three doses per week suffice to manage the vast majority of cases.[2]

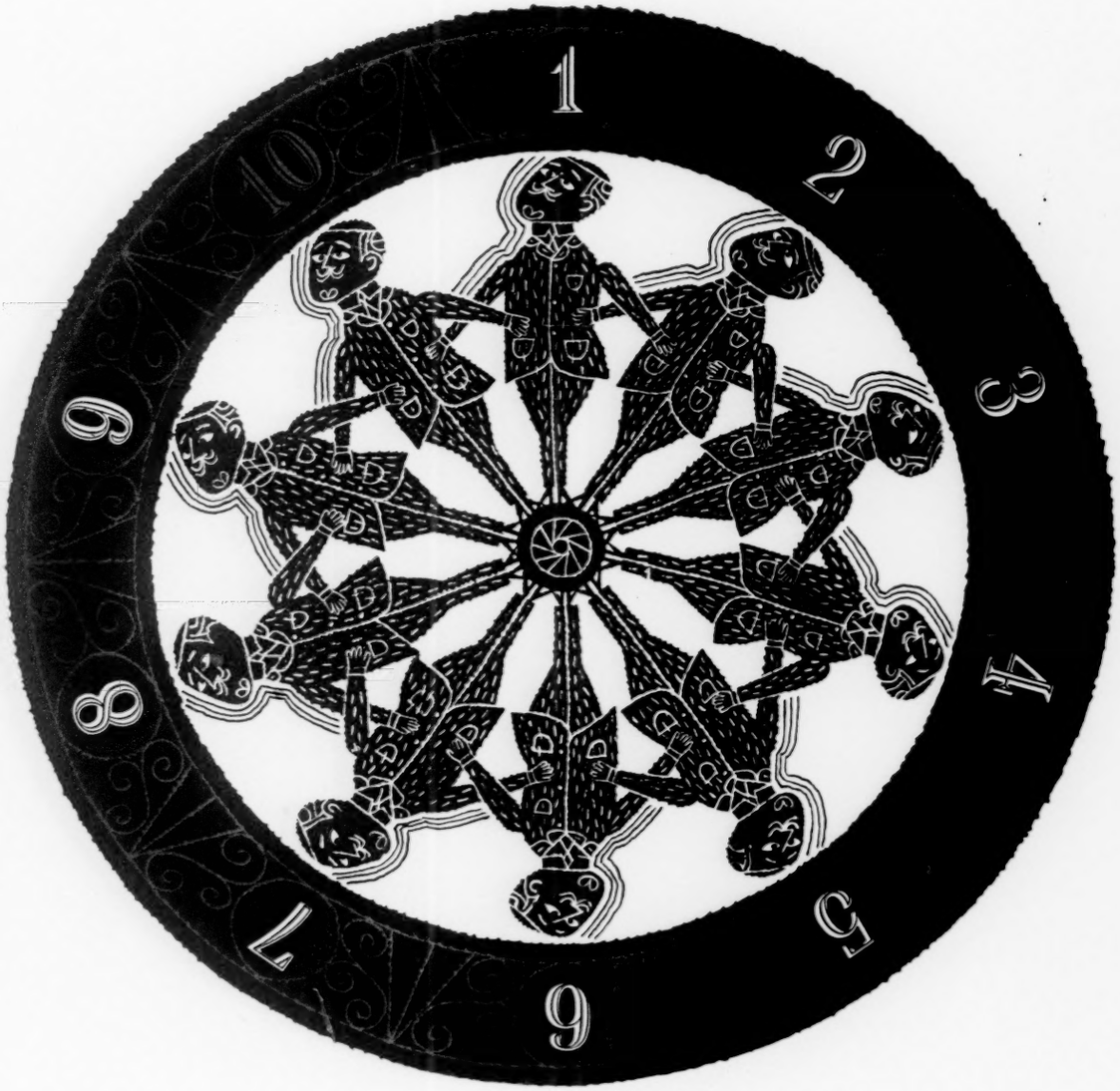
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Sustained control without side reactions.

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Copious diuresis without electrolyte imbalance.

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References:
(1) Stenger, E. G., et al.: Schweiz. med. Wchnschr. 89:1126, 1959. (2) Fuchs, M., Res: et al.: Current Therap. Research 2:11, January, 1960. (3) Ford, R. V.: Manuscript submitted for publication.

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Prescribe one ANTIVERT tablet (12.5 mg. meclizine; 50 mg. nicotinic acid) before each meal for relief of Meniere's syndrome, arteriosclerotic vertigo, labyrinthitis and vertigo of nonspecific origin.

Supplied: In bottles of 100 blue-and-white scored tablets. Prescription only.

References: 1. Menger, H. C.: Clin. Med. *4*:313 (Mar.) 1957. 2. Seal, J. C.: Eye Ear Nose & Throat Month. *38*:738 (Sept.) 1959.

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Give PANHEPRIN® initially

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for immediate control of coagulation

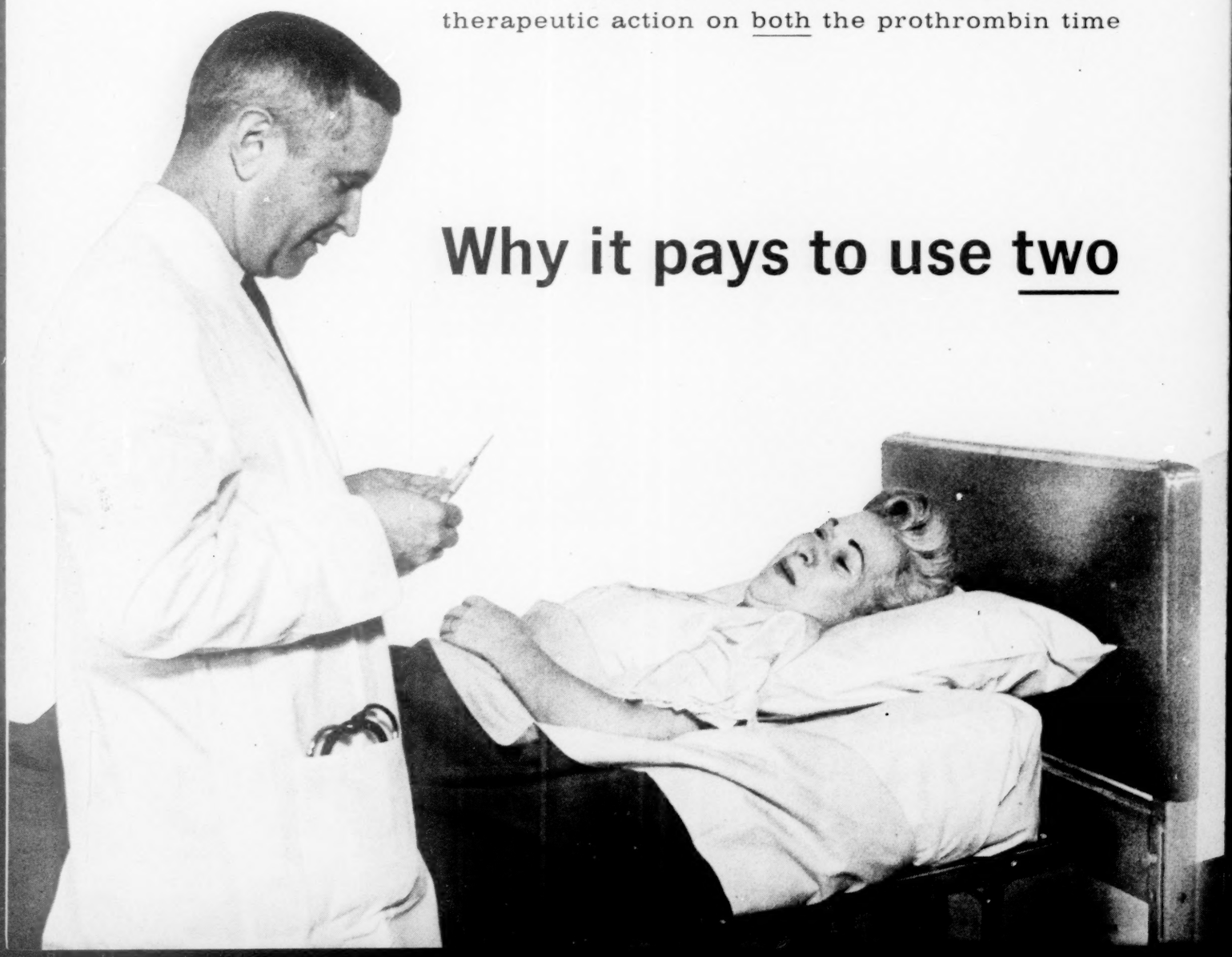
Combined treatment with heparin and a coumarin-like drug has been called "the closest approach to ideal anticoagulant therapy."[1] Parenteral and oral routes are used simultaneously. Here's how it works:

Start thrombosis and embolism patients on PANHEPRIN, given subcutaneously or intravenously. In this way you'll be using the agent of choice for rapid reduction of coagulability. It gives your patients immediate protection.

At the same time, start PANWARFIN orally. This coumarin-type agent will begin providing therapeutic prothrombin levels within 18 to 36 hours.

Both drugs may be continued for about ten days. The build-up period enables PANWARFIN to exert therapeutic action on both the prothrombin time

Why it pays to use two



Give **PANWARFIN**™ follow-up

(Warfarin Sodium, Abbott)

for predictable, low-cost oral maintenance

and the clotting time.

Thereafter, only oral PANWARFIN need be given. You'll find that maintenance is relatively easy, because PANWARFIN is predictable in effect. There's little daily variation in prothrombin times, and little juggling of dosage becomes necessary.

This combined therapy lets you provide maximum protection at the outset. Then it lets you shift to oral maintenance, to obtain added patient comfort and lower cost.

Abbott is the only company to offer you both heparin and warfarin products. Our literature gives full details on their combined and individual use. Ask your Abbott man for literature and information, or write us at North Chicago, Illinois.

1. Drugs of Choice 1960-1961 (Modell, W., Ed.); C. V. Mosby Co., St. Louis, 1960; p. 652.

At the physician's discretion, both products are often used singly. PANHEPRIN is suitable for both anticoagulant and lipemia clearing purposes, and is supplied in a handy disposable syringe (as well as vials and ampoules in concentrations from 1000 to 40,000 USP units/ml.) that makes even self-administration by the patient easy. And PANWARFIN, supplied in 5-, 10- and 25-mg. grooved tablets, may be prescribed alone for cases without urgency (e.g., chronic thrombophlebitis).

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exclusive oral
Sustained Medication*
Quinidine Gluconate (5 gr.)



for these good reasons[1-4]

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QUINAGLUTE DURA-TAB S.M. tablets maintain effective uniform blood levels around the clock, day and night.

better tolerated
because quinidine gluconate is ten times more soluble than the sulfate — and easier on the g.i. tract.

uniformly efficient
no let-down in plasma levels where arrhythmias tend to occur.

REFER TO PDR PAGE 893

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1. Bellet, S., Finkelstein, D., and Gilmore, H.: A.M.A Archives Int. Med. 100:750, 1957.
2. Bellet, S.: Amer. Heart J. 56:479, 1958.
3. Finkelstein, D.: Penn. Med. J. 61:1216, 1958.
4. Bellet, S.: Amer. J. Cardiology 4:268, 1959.

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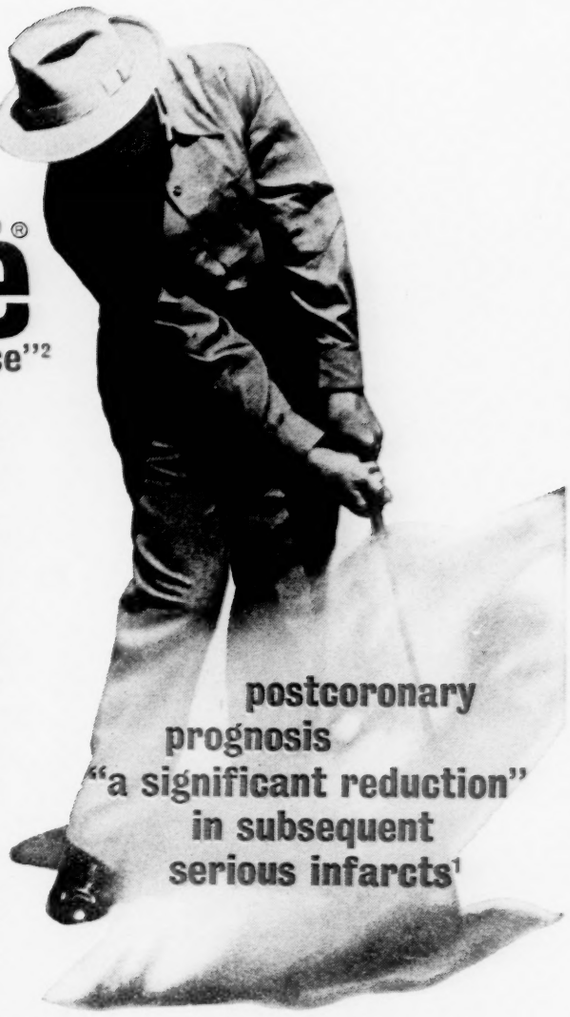
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10 cc. Vials, 0.08 Gm. Quinidine Gluconate per cc.

*U.S. Patent 2895881

Danilone (Phenindione, Schieffelin) ®

"the anticoagulant drug of choice"[2]



When DANILONE was administered to 33 postmyocardial infarct patients, only two thromboembolic episodes occurred in two patients in 565 patient-months. Five such episodes in 50 patient-months occurred in four patients who had stopped taking anticoagulants.[3]

"twice as fast in reaching therapeutic levels"[4] In 117 patients, the average time DANILONE required to attain therapeutic levels was less than 36 hours.[4] With bishydroxycoumarin, two to four days are required for equal effect.[5]

"a greater degree of control"[6] DANILONE held prothrombin activity in 200 patients to between 5 and 10 per cent of normal "with greater facility" than in another series where bishydroxycoumarin was used.[6] Generally, most satisfactory control can be obtained by dividing the dosage schedule into two 12-hour intervals.[3,6]

"a reputation for safety"[7] DANILONE, unlike the coumarin derivatives, has a short recovery period and no cumulative action.[5,8,9] As a result, DANILONE "... can be administered with reasonable safety in the presence of many conditions which would contraindicate the use of Dicoumarol."[6]

"a lack of toxic reactions"[3] Because of its extremely low incidence of toxicity,[2,4,9] and "... the apparent safety factor of a wide spread between therapeutic and toxic dose, it is felt that phenyldanedione [phenindione] is the anticoagulant drug of choice."[2]

"inexpensive"[5] DANILONE is the least expensive anticoagulant available today. Maintenance cost is as little as three cents a day. In addition, its stability of effect permits fewer prothrombin-time tests,[9] saving the patient additional costs.

Samples and literature available on request.

SUPPLIED: 50 mg. scored tablets. Bottles of 100 and 1,000.

References: 1. Pickering, G., et al.: Brit. M. J. 1:803 (Mar. 28) 1959. 2. McCabe, E. S., and Fittipoldi, J., Jr.: Am. Pract. & Digest Treat. 4:765, 1953. 3. Drinan, F. W., et al.: Am. Heart J. 53:284, 1957. 4. Harper, B. F., and Johnson, R.: J.M.A. Georgia 45:149, 1956. 5. Wood, J. E.; Beckwith, J. R., and Camp, J. L., III: J.A.M.A. 159:635 (Oct. 15) 1955. 6. Sise, H. S.; Maloney, W. C., and Guttas, C. G.: Am. Heart J. 53:132, 1957. 7. Newcomb, T. F.: New England J. Med. 260:545 (Mar. 12) 1959. 8. O'Connor, W. R.; Thompson, C. E., and Baker, L. A.: Quart. Bull. Northwestern Univ. M. School 26:193 (Fall) 1952. 9. Toohey, M.: Brit. M. J. 1:650 (Mar. 21) 1953.

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Naturetin

Squibb Benzydroflumethiazide

Naturetin c̄ K

Squibb Benzydroflumethiazide with Potassium Chloride

"...a safe and extraordinarily effective diuretic..."[1]

Naturetin — reliable therapy in edema and hypertension — maintains a favorable urinary sodium-potassium excretion ratio . . . retains a balanced electrolytic pattern:

"... the increase in urinary output occurs promptly ..."[1]

"... the least likely to invoke a negative potassium balance ..."[2]

"... a dose of 5 mg. of Naturetin produces a maximal sodium loss."[2]

"... an effective diuretic agent as manifested by the loss in weight ..."[3]

"... no apparent influence of clinical importance on the serum electrolytes or white blood count."[3]

"... no untoward reactions were attributed to the drug."[4]

Although Naturetin causes the least serum potassium depletion as compared with other diuretics, supplementary potassium chloride in Naturetin c̄ K provides added protection when treating hypokalemia-prone patients; in conditions where likelihood of electrolyte imbalance is increased or during extended periods of therapy.

Numerous clinical studies confirm the effectiveness[1-15] of Naturetin as a diuretic and antihypertensive — usually in dosages of 5 mg. per day.

■ the most potent diuretic, mg. for mg.—more than 100 times as potent as chlorothiazide ■ prolonged action — in excess of 18 hours ■ maintains its efficacy as a diuretic and antihypertensive even after prolonged or increased dosage use ■ convenient once-a-day dosage — more economical for patients ■ low toxicity — few side effects—low sodium diets not necessary ■ not contraindicated except in complete renal shutdown ■ *in hypertension*—significant lowering of the blood pressure. Naturetin may be used alone or with other antihypertensive drugs in lowered doses.

Supplied: Naturetin Tablets, 5 mg. (scored) and 2.5 mg. Naturetin c̄ K (5 c̄ 500) Tablets (capsule-shaped) containing 5 mg. benzydroflumethiazide and 500 mg. potassium chloride. Naturetin c̄ K (2.5 c̄ 500) Tablets (capsule-shaped) containing 2.5 mg. benzydroflumethiazide and 500 mg. potassium chloride.

SQUIBB



Squibb Quality—the Priceless Ingredient

References: 1. David, N. A.; Porter, G. A., and Gray, R. H.: Monographs on Therapy **5**:60 (Feb.) 1960. 2. Stenberg, E. S., Jr.; Benedetti, A., and Forsham, P. H.: Op. cit. **5**:46 (Feb.) 1960. 3. Fuchs, M.; Moyer, J. H., and Newman, B.E.: Op. cit. **5**:55 (Feb.) 1960. 4. Marriott, H. J. L., and Schamroth, L.: Op. cit. **5**:14 (Feb.) 1960. 5. Ira, G. H., Jr.; Shaw, D. M., and Bogdonoff, M. D.: North Carolina M. J. **21**:19 (Jan.) 1960. 6. Cohen, B. M.: M. Times, to be published. 7. Breneman, G. M., and Keyes, J. W.: Henry Ford Hosp. M. Bull. **7**:281 (Dec.) 1959. 8. Forsham, P. H.: Squibb Clin. Res. Notes **2**:5 (Dec.) 1959. 9. Larson, E.: Op. cit. **2**:10 (Dec.) 1959. 10. Kirkendall, W. M.: Op. cit. **2**:11 (Dec.) 1959. 11. Yu, P. N.: Op. cit. **2**:12 (Dec.) 1959. 12. Weiss, S.; Weiss, J., and Weiss, B.: Op. cit. **2**:13 (Dec.) 1959. 13. Moser, M.: Op. cit. **2**:13 (Dec.) 1959. 14. Kahn, A., and Greenblatt, I. J.: Op. cit. **2**:15 (Dec.) 1959. 15. Grollman, A.: Monographs on Therapy **5**:1 (Feb.) 1960.

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* Bishop, P. M. F.: *Brit. M. J.* 1:184, 1960.

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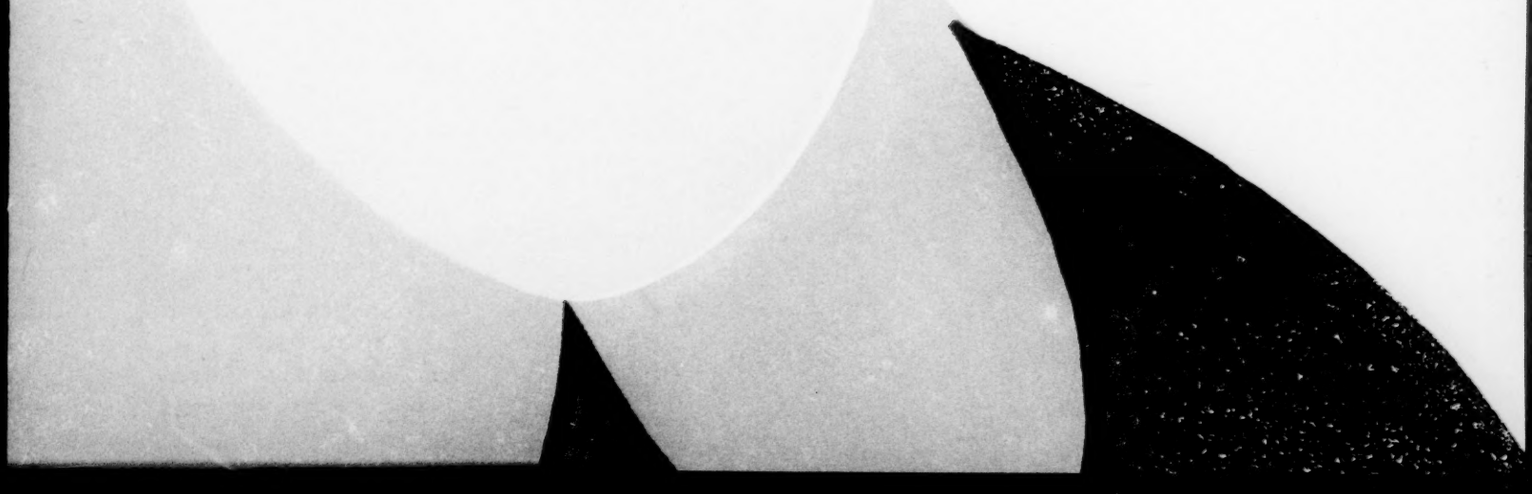
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safe and practical treatment of the postcoronary patient

A basic characteristic of the postcoronary patient, whether or not cholesterol levels are elevated, is his inability to clear fat from his blood stream as rapidly as the normal subject.[1-3] *Figure #1* graphically illustrates this difference in fat-clearing time by comparing atherosclerotic and normal subjects after a fat meal.[3]

"Slow clearers" gradually accumulate an excess of fat in the blood stream over a period of years as each meal adds an additional burden to an already fat-laden serum. As shown in *figure #2,* the blood literally becomes saturated with large fat particles, presenting a dual hazard to the atherosclerotic patient: the long-term danger of deposition of these fats on the vessel walls,[4] and the more immediate risk of high blood fat levels after a particularly heavy meal possibly precipitating acute coronary embarrassment.[5]

In *figure #3,* the test tube at the left contains lipemic serum, while the one at the right contains clear, or normal serum. If serum examined after a 12-hour fasting period presents a milky appearance, this is a strong indication that the patient clears fat slowly and is a candidate for antilipemic therapy in an effort to check a potentially serious situation.

'Clarin', which is heparin in the form of a *sublingual tablet,* has been demonstrated to clear lipemic serum.[2,6,7] Furthermore, a two-year study using matched controls resulted in a statistically significant reduction of recurrent myocardial infarction in 130 patients treated with 'Clarin'.[8]

'Clarin' therapy is simple and safe, requiring no clotting-time or prothrombin determinations. Complete literature is available to physicians upon request.

References: **1.** Anfinsen, C. B.: Symposium on Atherosclerosis, National Academy of Sciences, National Research Council Publication 338, 1955, p. 218. **2.** Berkowitz, D.; Likoff, W., and Spitzer, J. J.: Clin. Res. *7*:225 (Apr.) 1959. **3.** Stutman, L. J., and George, M.: Clin. Res. *7*:225 (Apr.) 1959. **4.** Wilkinson, C. F., Jr.: Annals of Int. Med. *45*:674 (Oct.) 1956. **5.** Kuo, P. T., and Joyner, C. R., Jr.: J.A.M.A. *163*:727 (March 2) 1957. **6.** Fuller, H. L.: Angiology *9*:311 (Oct.) 1958. **7.** Shaftel, H. E., and Selman, D.: Angiology *10*:131 (June) 1959. **8.** Fuller, H. L.: Circulation *20*:699 (Oct.) 1959.

Clarin*

(sublingual heparin potassium, Leeming)

Fig. 1

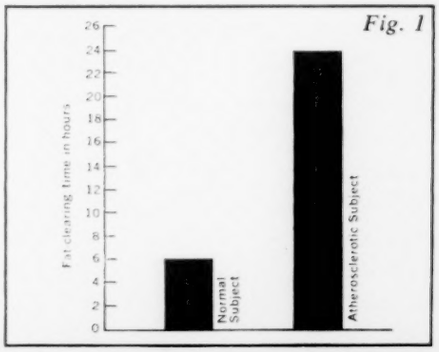


Fig. 2

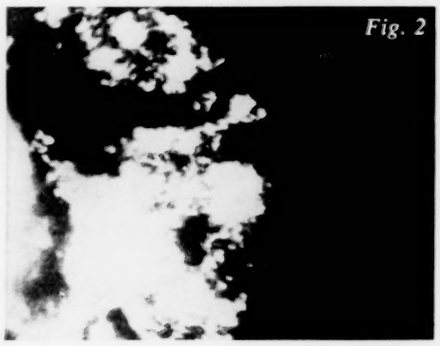
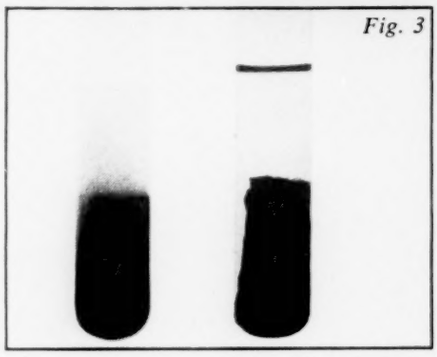


Fig. 3



Indication: For the management of hyperlipemia associated with atherosclerosis, especially in the postcoronary patient.

Dosage: After each meal, hold one tablet under the tongue until dissolved.

Supplied: 'Clarin' is supplied in bottles of 50 pink, sublingual tablets, each containing 1500 I.U. of heparin potassium.

*Registered trade mark. Patent applied for.

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1. Batterman, R. C.; Grossman, A. J.; Mouratoff, G. J., and Leifer, P.: A Clinical Re-evaluation of Daytime Sedatives, Scientific Exhibit, Annual AMA Meeting, San Francisco, Cal., June 23-27, 1958.

90% of anxious, agitated and apathetic office patients calmed without drowsiness and with normal drive restored...

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- PERMITIL does not potentiate barbiturates or non-barbiturate sedatives and can be used with impunity with such agents.

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SUPPLIED: Tablets, 0.25 mg., bottles of 50 and 500.

REFERENCES: 1. Ayd, F. J., Jr.: Current Therapeutic Research 1:41 (Oct.) 1959. 2. Recent compilation of case reports received by the Medical Department, White Laboratories, Inc.



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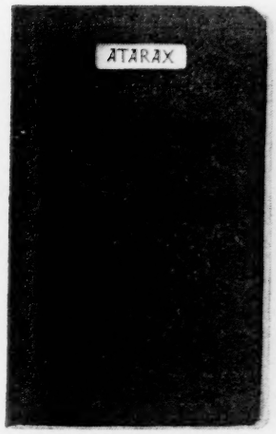
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Added frontiers of usefulness—antihistaminic; mildly antiarrhythmic; does not stimulate gastric secretion.

Special Advantages	Supportive Clinical Observation	...and for additional evidence
IN CHILDREN unusually safe; tasty syrup, 10 mg. tablet	"... Atarax appeared to reduce anxiety and restlessness, improve sleep patterns and make the child more amenable to the development of new patterns of behavior...." Freedman, A. M.: Pediat. Clin. North America **5**:573 (Aug.) 1958.	Bayart, J.: Acta paediat. belg. **10**:164, 1956. Ayd, F. J., Jr.: California Med. **87**:75 (Aug.) 1957. Nathan, L. A., and Andelman, M. B.: Illinois M. J. **112**:171 (Oct.) 1957.
IN ELDERLY PATIENTS well tolerated by debilitated patients	"... seems to be the agent of choice in patients suffering from removal disorientation, confusion, conversion hysteria and other psychoneurotic conditions occurring in old age." Smigel, J. O., et al.: J. Am. Geriatrics Soc. **7**:61 (Jan.) 1959.	Settel, E.: Am. Pract. & Digest Treat. **8**:1584 (Oct.) 1957. Negri, F.: Minerva med. **48**:607 (Feb. 21) 1957. Shalowitz, M.: Geriatrics **11**:312 (July) 1956.
IN ALLERGIC PATIENTS useful adjunctive therapy for asthma and dermatosis; particularly effective in urticaria	"All [asthmatic] patients reported greater calmness and were able to rest and sleep better... and led a more normal life.... In chronic and acute urticaria, however, hydroxyzine was effective as the sole medicament." Santos, I. M., and Unger, L.: Presented at 14th Annual Congress, American College of Allergists, Atlantic City, New Jersey, April 23-25, 1958.	Eisenberg, B. C.: J.A.M.A. **169**:14 (Jan. 3) 1959. Coirault, R., et al.: Presse méd. **64**:2239 (Dec. 26) 1956. Robinson, H. M., Jr., et al.: South. M. J. **50**:1282 (Oct.) 1957.
IN HYPEREMOTIVE ADULTS does not impair mental acuity	"... especially well-suited for ambulatory neurotics who must work, drive a car, or operate machinery." Ayd, F. J., Jr.: New York J. Med. **57**:1742 (May 15) 1957.	Garber, R. C., Jr.: J. Florida M. A. **45**:549 (Nov.) 1958. Menger, H. C.: New York J. Med. **58**:1684 (May 15) 1958. Farah, L.: Internat. Rec. Med. **169**:379 (June) 1956.

SUPPLIED: Tablets, 10 mg., 25 mg., 100 mg.; bottles of 100. Syrup (10 mg. per tsp.), pint bottles. Parenteral Solution: 25 mg./cc. in 10 cc. multiple-dose vials; 50 mg./cc. in 2 cc. ampules.

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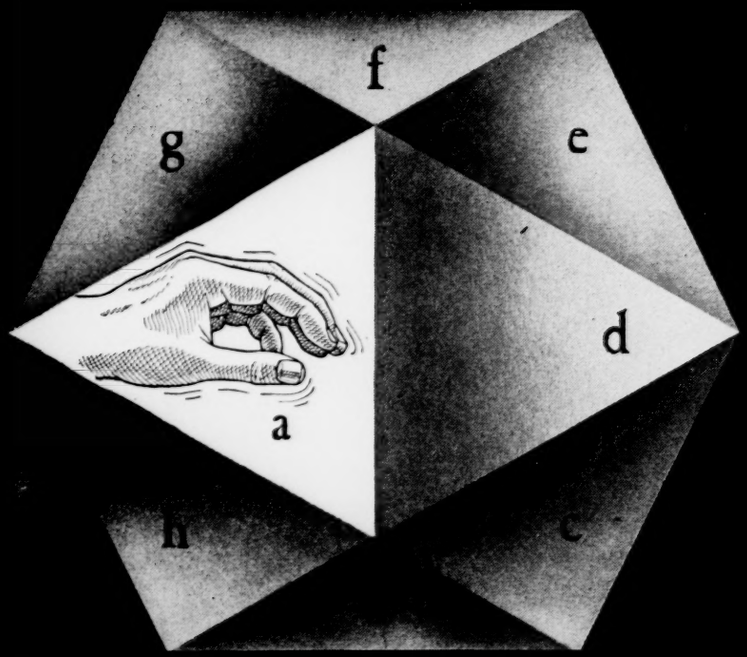
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Dosage: usually 1 tablet (50 mg.) t.i.d. When used in combination, dosage should be correspondingly reduced.

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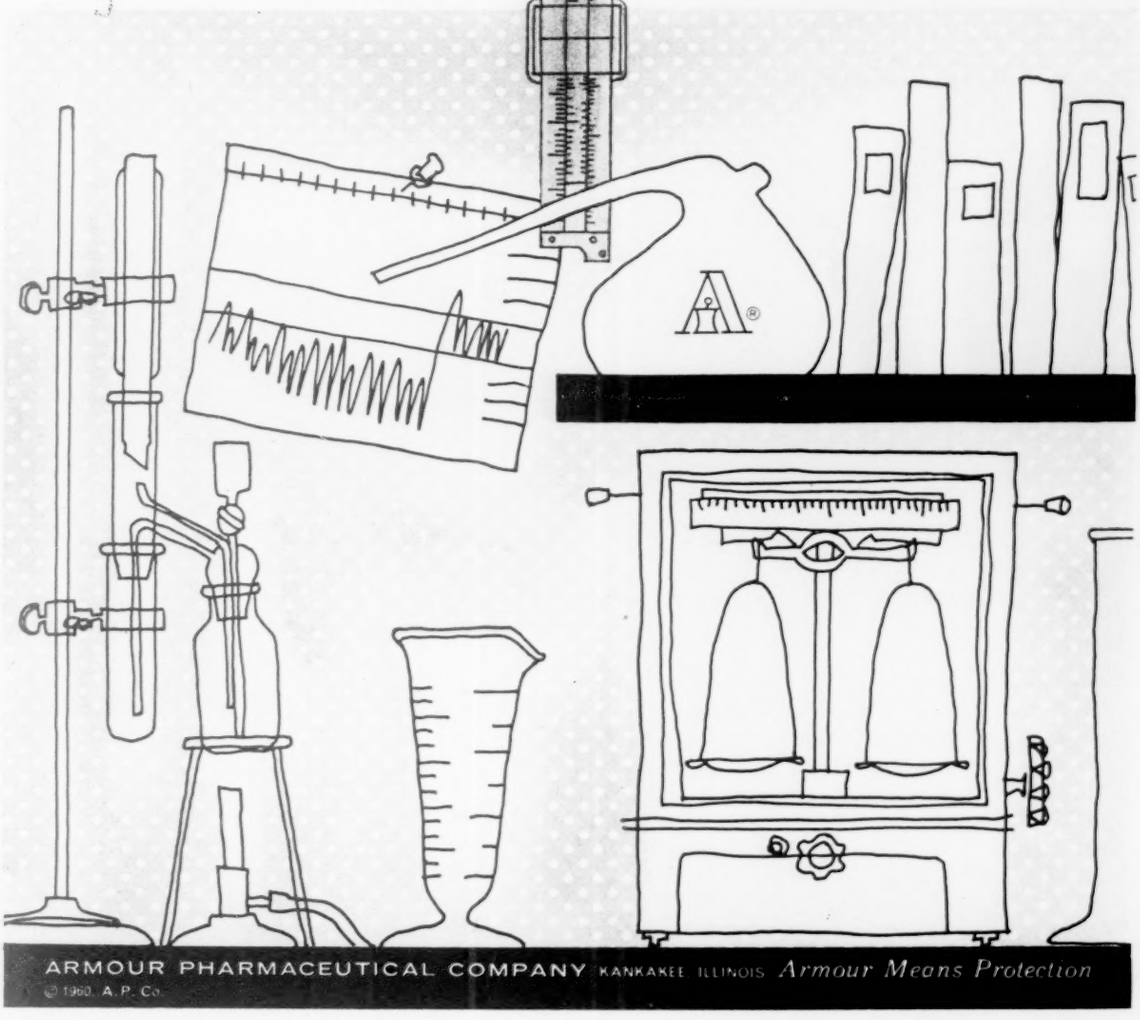
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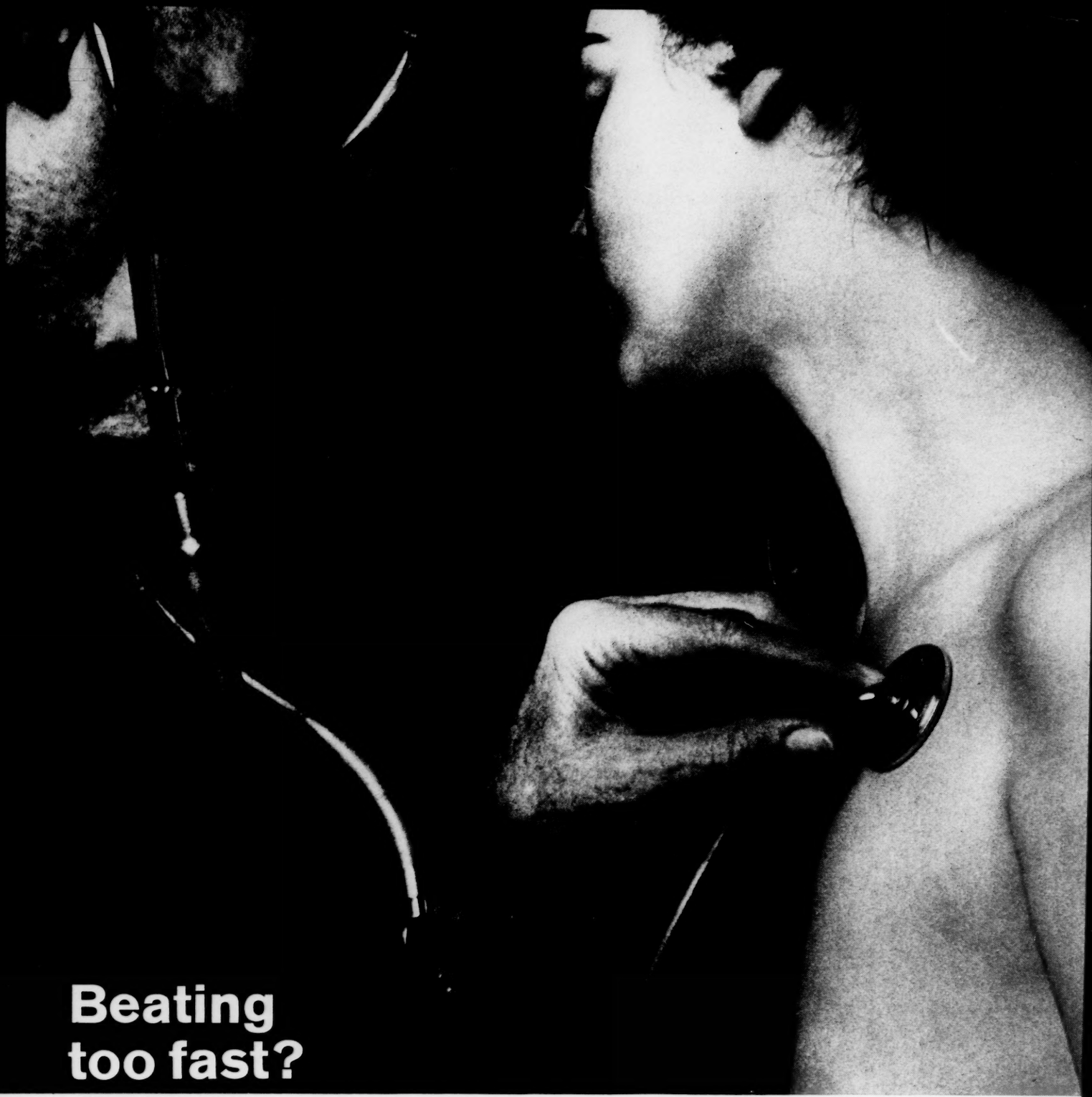
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Lichtman[1,2] used Trancopal to treat patients with low back pain, stiff neck, bursitis, rheumatoid arthritis, osteoarthritis, trauma, and postoperative muscle spasm. He noted that Trancopal produced satisfactory relief in 817 of 879 patients (excellent results in 268, good in 448 and fair in 101).

Gruenberg[3] prescribed Trancopal for 70 patients with low back pain and observed that it brought marked improvement to all. "In addition to relieving spasm and pain, with subsequent improvement in movement and function, Trancopal reduced restlessness and irritability in a number of patients."[3] In another series, Kearney[4] reported that Trancopal produced relief in 181 of 193 patients suffering from low back pain and other forms of musculoskeletal spasm.

Trancopal enables the anxious patient to work or play. According to Gruenberg, "In addition to relieving muscle spasm in a variety of musculoskeletal and neurologic conditions, Trancopal also exerts a marked tranquilizing action in anxiety and tension states."[3] Kearney[4] found "... that Trancopal is the most effective oral skeletal muscle relaxant and mild tranquilizer currently available."

Side effects are rare and mild. "Trancopal is exceptionally safe for clinical use."[3] In the 70 patients with low back pain treated by Gruenberg,[3] the only side effect noted was mild nausea which occurred in 2 patients. In Lichtman's group, "No patient discontinued chlormethazanone [Trancopal] because of intolerance."[1]

How Supplied: Trancopal Caplets®
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100 mg. (peach colored, scored), bottles of 100.

Dosage: Adults, 200 or 100 mg. orally three or four times daily. Relief of symptoms occurs in from fifteen to thirty minutes and lasts from four to six hours.

References: 1. Lichtman, A. L.: *Kentucky Acad. Gen. Pract. J.* 4:28, Oct., 1958. 2. Lichtman, A. L.: Scientific Exhibit, Internat. Coll. Surgeons, Miami Beach, Fla., Jan. 4-7, 1959. 3. Gruenberg, Friedrich: *Current Therap. Res.* 2:1, Jan., 1960. 4. Kearney, R. D.: *Current Therap. Res.* 2:127, April, 1960.

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Coolidge, C. W.; Glisson, C. S., and Smith, A. S.: J.M.A. Georgia 48:167, 1959.

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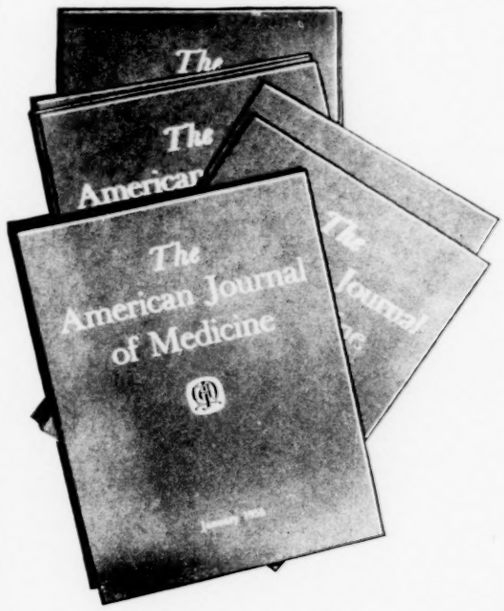
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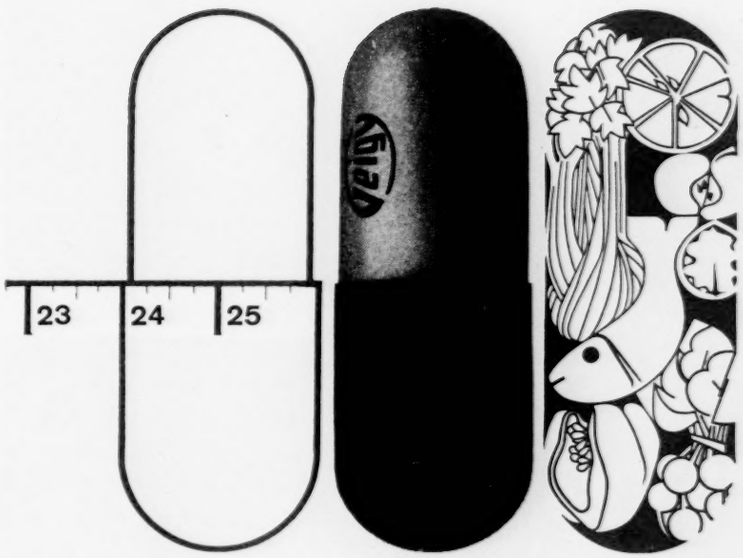
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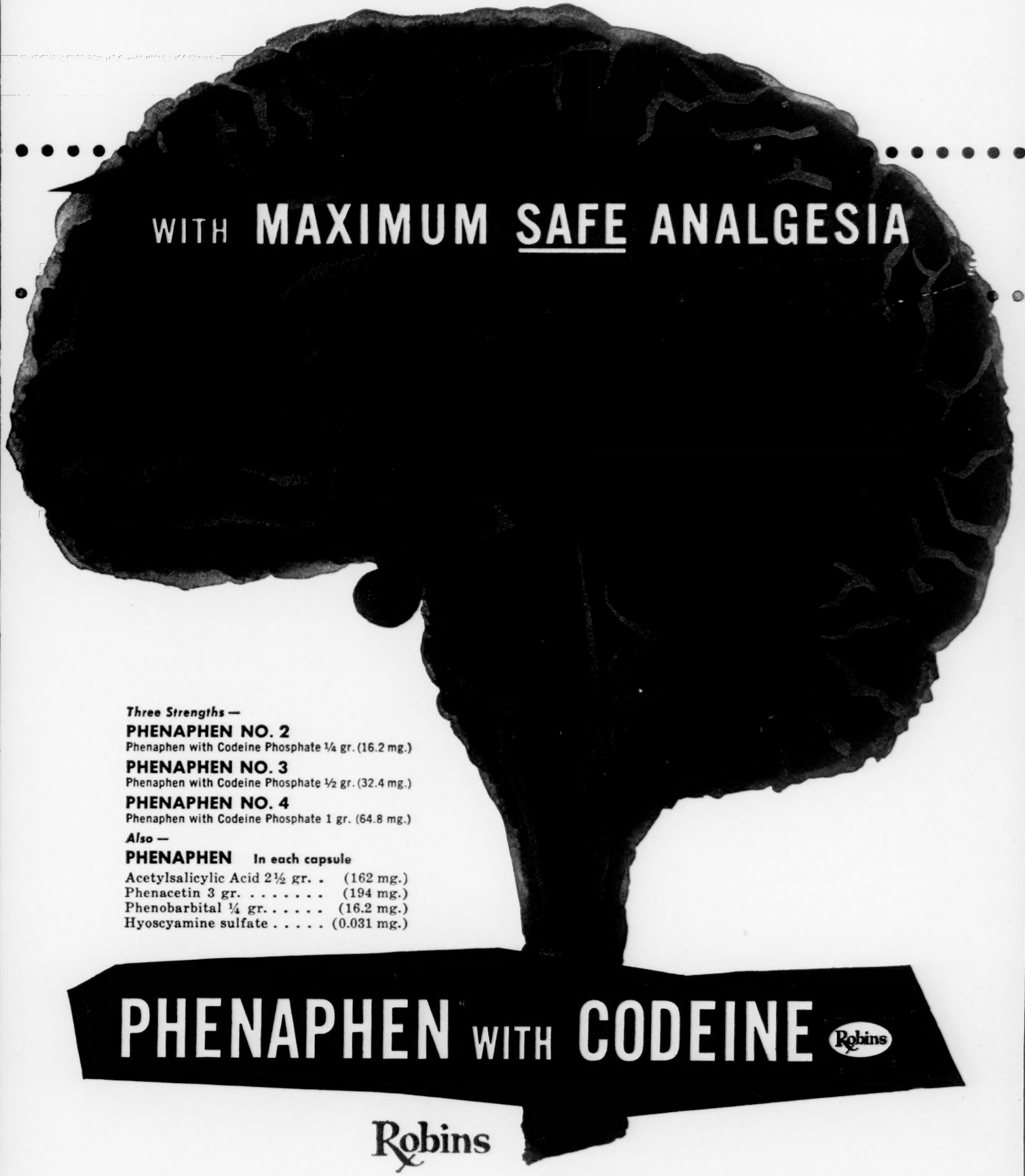
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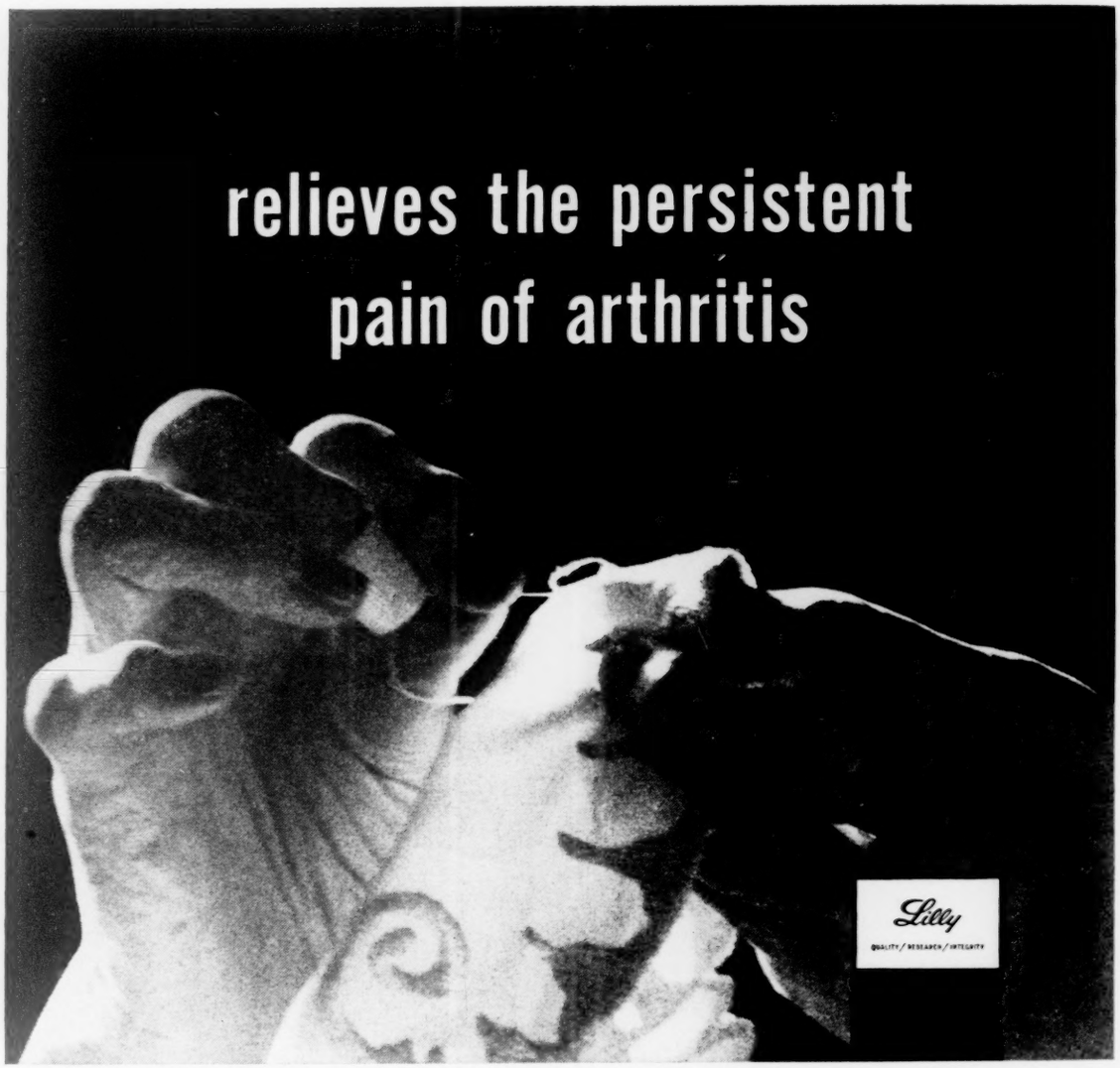
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The American Journal of Medicine

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Editorial

Plasma Insulin in Man

THE study and interpretation of disease processes that are associated with abnormalities of insulin secretion or insulin utilization have been hampered by the lack of an acceptable assay for insulin in plasma. There has recently been a surge of interest in the measurement of plasma insulin, and although much of the data is yet of a preliminary nature, an attempt to correlate the activities in this field appears to be justified at this time.

Even were it possible to extract plasma insulin completely free of other proteins, the direct chemical determination of insulin derived from a reasonable amount of plasma would be prohibited by the low concentration of hormone present. Therefore, insulin in plasma must be measured indirectly in some biologic or chemical system, by comparison of the effects of plasma with those of known amounts of crystalline insulin. Since other plasma substances may exert insulin-like activity in certain systems, it is important to demonstrate, in each system employed, that insulin-like activity behaves like insulin in at least several respects; for example, that plasma insulin-like activity is absent when insulin is known to be absent, as in completely depancreatized animals, and that substances known to inhibit or inactivate insulin do likewise with plasma insulin-like activity.

Methods promising to be useful may be divided into 3 main categories: (1) *in vivo* bioassay—measurement of the reduction in blood sugar induced by injection of plasma into animals sensitized to the action of small amounts of insulin; (2) *in vitro* bioassay—measurement of the effect of plasma on some aspect of glucose metabolism in excised muscle or fat tissue; and (3) immunoassay. A summary of methods and estimated plasma insulin concentrations is given in Table 1. It is evident that sensitivities and results with different methods vary widely.

Circulating insulin was first successfully demonstrated by *in vivo* bioassay. Whereas adrenalectomized rats or mice [*1,2*] were found not to be suitable for assay of blood insulin, Gellhorn et al. [*2*] discovered that AdemH rats (see Table 1 for abbreviations) were sufficiently sensitive to insulin to permit an estimate of 200 microunits/ml. whole blood for the insulin concentration in man two and a half hours after a meal. It was also demonstrated that the hypoglycemic effect of insulin was not altered when insulin was injected together with blood. Anderson et al. [*3*] observed that AdemDH rats were sensitive to as little as 125 microunits insulin but they were unable to detect insulin in plasma from fasting rats. Bornstein and Lawrence [*4*], employing ADH rats [*5*], reported that plasma insulin concentrations two hours after a glucose feeding averaged about 340 microunits/ml. in normal subjects and 100 to 320 microunits/ml. in patients with diabetes not subject to ketosis. However, Peden [*6*] and Randle [*7*] were unable to confirm the suitability of ADH rats since in their hands a large fraction failed to survive the operative procedures and those that survived were too unstable to serve for insulin assay. More recently, Baird and Bornstein [*8*], employing AD mice, reported that normal fasting plasma extracted with acid ethanol-N-butanol-toluene contains about 1,000 microunits insulin/ml. but these values were estimated

TABLE 1*

Data	Sensitivity (minimum detected) (microunits)	Estimated Mean (and range) Human Plasma Insulin Concentrations (microunits/ml.)		Reference
		Fasting	After Glucose or Feeding	
A. In Vivo Bioassay				
Preparation				
A rat	2,000	...	...	Hemmingsen et al. [1]
AdemH rat	300	...	200 (whole blood)	Gellhorn et al. [2]
AdemDH rat	125	Not detectable (rat)	...	Anderson et al. [3]
ADH rat	50	...	...	Bornstein [5]
ADH rat	50	...	340	Bornstein, Lawrence [4]
ADH rat	<2,000	...	Not detectable	Randle [16]
DH rat	>2,500	...	...	Beigelman et al. [50]
Intact mice	1,000	Detectable in serum fractions	Detectable in serum fractions	Beigelman [51]
DH mice	250	...	...	Anderson et al. [9]
DH mice	1,000–2,000	...	Detectable in concentrated protein precipitates but not in plasma	Goetz et al. [52]
AD mice	†	(800–1,150)	(1,700–3,700)	Baird, Bornstein [8]
B. Rat Diaphragm Bioassay (Measurement of Glucose Uptake)				
Dilution of serum or plasma employed				
Undiluted	Variable, 10–500	(62–625)	...	Groen et al. [20]
Undiluted	10	64(40–80)	(130–800)	Vallance-Owen et al. [11,12]
Undiluted	20 (10 microunits/ml.)	59(11–240)	573(71–>1,000)	Seltzer, Smith [13]
Undiluted	20 (10 microunits/ml.)	(15–60)	<300	Seltzer, Smith [14]
Undiluted	‡	10(0.1–44)	...	Field, J. [14]
1:1.1–1:2	30	(100–900)	...	Willebrands et al. [10]
1:5 –1:50	30	(400–4,600)	13,500(9,000–22,000)	Willebrands et al. [10]
1:4	100	...	...	Randle [16]
1:5	100	(100–3,000)	Not detectable	Willebrands et al. [15]
1:2 (measured glycogen synthesis)	5,000	Not detectable		Perlmutter et al. [53]
Mouse Diaphragm Bioassay				
1:3	30	...	...	Oyama, Grant [19]
1:3	30	...	24	Oyama, Hagen, H. M., Grant [14]
C. Rat Epididymal Fat Pad Bioassay				
Dilution of serum or plasma employed				
Undiluted	20 (10 microunits/ml.)	(50–350)	...	Martin et al. [28]
Undiluted	20 (10 microunits/ml.)	250(31–900)	3–6 fold increase	Renold et al. [14]
Undiluted	...	247(1–1,000)	3–4 fold increase	Owen, J. A., Jr., Schocken, V. [14]
1:2	...	(130–680)	...	Pfeiffer et al. [29]
1:10	20 (10 microunits/ml.)	...	Detectable	Beigelman [54]
1:5 –1:10	...	(300–1,200)	Negligible change	Leonards [14]
D. Immunoassay				
Immunologic system				
Inhibition of hemolysis of sensitized erythrocytes	2,800 (beef insulin)	...	...	Arquilla, Stavitsky [33]
Skin sensitivity	4 (beef insulin)	Not detectable	...	Loveless [34]
Competitive inhibition of binding of labeled insulin	0.25 (human insulin)	21(0–66)	140(30–300)	Yalow, Berson [43–45]
Competitive inhibition of binding of labeled insulin	20 (beef insulin)	Not detectable	31	Grodsky, Forsham [46]

* A = adrenalectomized. Adem = adrenodemedullated. D = diabetic (alloxan-induced). H = hypophysectomized. ... = not reported.
† Lowest concentration used for standards 1,000 microunits/ml.
‡ Lowest standard, 10 microunits/ml.; lower values obtained by extrapolation.

from data falling below the standardized range; after feeding, plasma concentrations rose about threefold. Plasma from four of six patients with diabetes showed insulin concentrations ranging from 2,300 to 7,350 microunits/ml. (it was not stated whether fasting or fed). The discrepancy between these concentrations and those found earlier by Bornstein and Lawrence using ADH rats [*4*] was explained on the basis of removal of insulin antagonists in the extraction procedure, but values on non-extracted plasma were not reported in the later paper [*8*].

The *in vivo* technics have the distinct advantage of measuring the blood sugar response in the whole animal, and assay by these means may therefore reflect the over-all effect of plasma insulin, together with its inhibitors and imitators. However, the sensitivity is generally not very great (Table 1A) and the methods are extremely onerous. A large number of specially prepared animals is required, the numerous operations to which the animals are subjected exact a variable but usually high mortality rate, and the animals that survive may be useful for only a single assay [*8*], or at most six assays [*9*]. As many as twelve to sixteen animals [*8,9*] may be required for the assay of a single plasma sample if the results are to be statistically valid. Therefore, most investigators have preferred *in vitro* assays for ease and economy.

The wide variability in estimates of plasma insulin concentration by the diaphragm technic (Table 1B) is in part related to the plasma dilution at which the measurements are performed. It is generally agreed [*7,10*] that dilution of serum or plasma increases markedly the estimate of plasma insulin concentration with the diaphragm technic. Assaying undiluted plasma, Vallance-Owen et al. [*11,12*] find that the insulin concentration in the plasma of normal fasting subjects averages about 65 μU/ml. and that the concentration increases approximately tenfold following administration of 50 gm. glucose orally. Seltzer and Smith [*13*], employing the same technic as Vallance-Owen et al., reported similar findings in normal subjects and, more recently [*14*], somewhat lower values. Adult subjects with diabetes, responsive to tolbutamide, showed average fasting levels (103 microunits/ml.) above normal but an average concentration (453 microunits/ml.) slightly below normal one hour after administration of glucose [*13*]. Willebrands and Groen [*15*], using serum diluted 1:5, reported a range of 100 to 3,000 microunits/ml. in the plasmas of fasting normal subjects (over half the values exceeding 1,000 microunits/ml.) but in plasma diluted 1:2 or less, fasting levels exceeded 400 microunits/ml. in only two of six cases [*10*]. Randle [*16*], assaying plasma diluted 1:4, has found still higher levels, with activity corresponding to 9,000 to 22,000 microunits/ml. two and a half hours after administration of glucose.

Another possible explanation for the discordant results reported by different workers applying apparently similar technics lies in the well known tendency for insulin, when present in low concentration in the absence of carrier protein, to adsorb strongly to glassware [*17,18*]. Randle [*7*] and Willebrands et al. [*10*] have studied the effects of albumin and other proteins added to the medium bathing the diaphragm, but it is not evident in these reports (or in many others) that carrier protein was added to the original insulin dilutions used as standards. Loss of insulin from these solutions could give rise to markedly exaggerated estimates of plasma insulin concentration on comparison with the effects of the standard solutions.

The lowest estimates that have come to our attention are those of Field [*14*] who observed an average fasting plasma concentration of 10 μU/ml. (range 0.1 to 44 microunits/ml.) and of Oyama, Hagen and Grant [*14*] who found the serums of *non-fasted* subjects to contain an average of only 24 microunits/ml. by measuring glucose uptake in *mouse* diaphragm [*19*]. The latter workers also noted that normal serum inhibits the activity of added insulin in this system and they therefore prepared their insulin standards with serum [*14*].

In support of the conclusion that plasma insulin-like activity in the rat diaphragm system is primarily due to insulin *per se* is evidence that insulin-like activity is absent in depancreatized dogs [*20*], cats [*21*] and man (Seltzer and Smith [*14*]), and is destroyed by sulfhydryl agents such as cysteine or reduced glutathione [*11,16,20*] which are known to abolish the hormonal effects of insulin [*22*]. However, some workers have concluded that the diaphragm responds to other plasma substances as well as to insulin. Randle found that plasmas containing insulin-like activity in the diaphragm assay corresponding to 13,000 [*16*] and 41,000 microunits [*23*] were without effect on the blood sugar of DH rats whereas 2,000 microunits of insulin produced a significant hypoglycemia in these animals.

Randle has suggested [*7,23*], on the basis of studies in hypopituitarism and acromegaly, that the insulin-like activity of human plasma as assayed by the rat diaphragm technic is influenced by the activity of the pituitary gland. Although this would not exclude insulin itself as the active agent, Bornstein and Hyde [*24*] have reported that human pituitary extract accelerates glucose utilization by rat diaphragm *in vitro*. The studies of Levine, Goldstein and associates [*25,26*] have demonstrated that muscular exercise induces a discharge of some hormonal substance into the plasma which promotes the intracellular penetration of galactose and other insulin-sensitive sugars into muscle throughout the body in the complete absence of insulin, and that a hormonal substance derived from the liver is necessary for optimal glucose utilization by the lower extremities in the presence of insulin [*27*].

Martin, Renold and Dagenais [*28*], by measuring the oxidation of glucose-1-C^{14} to $C^{14}O_2$ by rat epididymal adipose tissue in the presence of undiluted serum, have estimated insulin-like activity in fasting normal subjects to be 50 to 350 microunits/ml. More recently Renold and coworkers [*14*] have found an average fasting value of 250 microunits/ml., with a range of 31 to 900 microunits/ml. but rarely above 500 microunits/ml. Pfeiffer et al. [*29*], employing the same technic, report that serum diluted 1:2 yields higher and more constant insulin concentrations than undiluted serums, and Leonards [*14*] has also observed an increase in plasma insulin-like activity on dilution of the plasma. However, the "dilution" effect has not been observed by Renold et al. [*14*] or by Owen and Schocken [*14*].

The studies of Leonards [*30*] have raised a serious question as to what part of the insulin-like effect of plasma on fat tissue is due to insulin itself. Leonards [*30*] has found that the insulin-like activity in plasma persists after total pancreatectomy when tested on the rat epididymal fat pad (a finding confirmed recently by R. H. Egdahl [*14*]) and that serum from guinea pigs immunized against insulin neutralizes the action of insulin in this system but has little or no effect on the insulin-like activity of human serum. Renold et al. [*14*] also found that insulin-like activity is incompletely inactivated by antiserum to insulin. Although serum insulin-like activity in the fat pad is inhibited by reduced glutathione (Renold et al. [*14*]), inhibition is incomplete (Owen and Schocken [*14*]). Furthermore, Leonards [*14*] has observed that insulin-like activity of plasma when measured by incorporation of C^{14} into glycogen of the fat pad is considerably lower than that estimated from the rate of $C^{14}O_2$ production, and that there is little increase in insulin-like activity of the serum after administration of glucose, only two of eight subjects responding and then with only a 50 per cent increase in insulin-like activity. The latter observation is contrary to the experience of Renold et al. [*14*] and Owen and Schocken [*14*] who report at least a threefold increase after glucose.

The influence of dilution of serum or plasma on estimated insulin concentrations in rat epididymal fat pad and in rat diaphragm assays has been attributed to the presence of inhibitory substances, the effects of which are diluted out more rapidly than those of insulin [*10,29*]. Groen et al. [*31*] have demonstrated that as little as 10^{-2} μg. of epinephrine counteracts the effect of 500 microunits insulin on uptake of glucose by the rat diaphragm. Willebrands and Groen [*36*] have reviewed the inhibitory effects of steroid hormones. There have also been many reports of incompletely characterized insulin inhibitors in the serum of normal and diabetic subjects (see reference 32 for review). In view of these reports and the interpretation of some workers [*10,29*] that plasma dilution removes the effects of insulin inhibitors in normal plasma, it is strange that the recovery of insulin added to plasma has not been investigated in most studies with *in vitro* systems, and when examined, the recovery is often [*11,16,21*] although not always (Oyama, Hagen and Grant [*14*]) reported to be quantitative.

In view of the uncertainties regarding the specificity of bioassay methods for insulin *per se*, an immunologic assay should be a welcome addition to the stock in trade available for determination of plasma insulin. Arquilla and Stavitsky [*33*], measuring hemagglutination of insulin-sensitized erythrocytes by insulin antiserums and inhibition of the reaction in the presence of excess insulin, and Loveless [*34*], using skin sensitivity technics, have devised quantitative assays for insulin based on immunologic principles, but these methods have not been developed with sufficient sensitivity for the assay of plasma insulin. However, the application of isotopic methods has made possible an exquisitively sensitive immunoassay for insulin. The binding of insulin-I^{131} to insulin

antibodies with the formation of non-precipitating complexes, the displacement of labeled insulin from insulin-I^{131}-antibody complexes by unlabeled insulin, and the quantitative relationship revealed in decrease of the ratio $\frac{\text{Bound insulin}}{\text{Free insulin}}$ (B/F) with increase in insulin concentration [*35*] have formed the basis for the measurement of microquantities of insulin [*36,37*]. Insulin-I^{131} bound to antibody and "free," unbound insulin-I^{131} are easily and completely separated by paper chromatoelectrophoresis technics [*35*]. Other separation methods may also be used, e.g., the precipitation of insulin-antibody complexes with antiserums directed against antibody globulins, as described by Skom and Talmage [*38*]. The assay of animal insulins such as pork or beef insulin, using human antiserums against these insulins, is capable of detecting as little as a microunit of animal insulin [*37*]. Following intravenous administration of insulin, the plasma disappearance of unlabeled beef insulin measured by immunoassay was shown to be in excellent agreement with the plasma disappearance of I^{131}-labeled insulin determined by plasma radioactivity measurements [*37*], and insulin concentrations in beef pancreas extracts determined by immunoassay and by mouse convulsion assay* were almost identical [*39*]. Grodsky and Peng [*40*] estimated the insulin content of mouse pancreas extracts using the immunoassay method, but employed beef insulin as a standard for mouse insulin and did not compare their results with a bioassay method. In view of the demonstrated species-specificity for the reactions of different mammalian insulins with specific antiserums [*41,42*], the use of insulin of one species as a standard for that of another requires the demonstration that the two insulins react identically in the immunologic system employed.

Although human insulin also was found to react with human antibeef, pork insulin serum [*37,41,42*] its reaction in this system proved too weak for expedient assay of human plasma insulin concentration. However, guinea pig antiserums against beef insulin proved eminently satisfactory for this purpose [*39*], permitting the measurement of as little as 0.25 microunits human insulin [*43*]. In this system plasma and standard human insulin solutions are compared in their ability to reduce the binding of beef insulin-I^{131} to antibody in guinea pig antibeef insulin serum [*39,43*]. It has been demonstrated that endogenous insulin in plasma measured by immunoassay is destroyed by cysteine and is adsorbed by cellulose to the same extent as insulin-I^{131} added *in vitro* [*44*]. Human insulin added to plasma is recovered quantitatively and, on dilution of plasma, a proportional decrease in endogenous insulin concentration is measured by immunoassay [*44,45*]. As might be expected in an immunologic system, the reaction is not only specific for insulin but also shows a high degree of species specificity [*41,42*]. Furthermore, the A and B chains of the split insulin molecule are no longer reactive [*42*].

Plasma insulin concentrations in man determined by immunoassay are among the lowest values reported. Fasting plasma concentrations in thirty subjects without diabetes and in thirty-eight patients with maturity-onset diabetes [*44*] did not differ significantly and averaged about 20 to 30 microunits/ml. (range 0 to 70 microunits/ml.), confirming earlier results in a smaller series of patients [*45*]. During a standard oral 100 gm. glucose tolerance test, insulin concentrations rose to a peak, averaging 140 microunits/ml., in a half hour or one hour in most subjects without diabetes, but peak concentrations, often exceeding 300 microunits/ml., were not achieved until two hours in the majority of those with diabetes [*44,45*]. The average insulin concentration during the two-hour period was approximately 25 per cent greater in the patients with diabetes than in control subjects [*44*]. Fasting insulin concentrations were elevated above 110 microunits/ml. in five of seven patients with functioning islet cell tumors [*44*].

Grodsky and Forsham [*46*] have measured insulin concentrations in extracts of plasma and, employing a salt precipitation method for separation of bound and free insulin, reported a sensitivity of 20 microunits/ml. using beef insulin as a standard for endogenous human insulin. However, it is probable that their sensitivity for detection of human insulin would be less than that for beef insulin by a factor of 2 or more, since it has been shown that human insulin reacts less strongly than beef insulin in guinea pig antibeef insulin serums [*44,45*]. This difference in reaction of the two insulins may explain the inability of Grodsky and Forsham [*46*] to detect insulin in the plasma of most fasting subjects and the small rise they observed in estimated insulin concentrations (mean, about

* The mouse convulsion assays were performed by Dr. R. Schucher, Montreal, Canada.

30 microunits/ml.) following glucose feeding in five subjects. The great sensitivity of the immunoassay employing insulin-I^{131} is sacrificed if advantage is not taken of the most sensitive available methods. With paper strip technics, routine determination of plasma insulin concentration requires only 10 to 20 μL. plasma, although 200 μL. may be used if desired. With appropriately diluted antiserums and high specific activity insulin-I^{131} preparations a 50 per cent reduction in the B/F ratio for beef insulin-I^{131} is produced by human insulin at a concentration of about 15 microunits/ml. [*44*] and precision measurements are possible with absolute amounts of insulin below 1 microunit [*43–45*].

A definitive evaluation of the merits and disadvantages of each assay method would be premature at this time. However, it would appear that methods showing fasting plasma insulin concentrations in the range 500 to 1,000 microunits/ml. or higher are defective, since these values cannot readily be resolved with established facts relating to the biology of insulin. Unless the properties of endogenous plasma insulin differ radically from those of exogenous insulin in respect to space of distribution (one to two times extracellular volume) and turnover rate (2 per cent/minute) [*35,37*], a secretion rate of 300 to 700 units/day would be required just to maintain these high fasting concentrations. Furthermore, in order to explain the absence of sustained marked hypoglycemia under such conditions, it would be necessary to postulate that endogenous human insulin has a much lower biologic effectiveness than exogenously administered animal insulins in man but not in the assay system yielding these estimates.*

In light of these considerations it would appear that the most "reasonable" estimates are those derived from the diaphragm assay with undiluted plasma, *in vivo* assays with non-extracted plasma, and immunoassay. To explain the dilution effect it may be suggested that plasma contains substances which augment (rather than inhibit) the effect of insulin on the diaphragm and that, in undiluted plasma, insulin itself is primarily responsible for glucose uptake but that the other substances play a relatively greater role when insulin concentration is lowered by plasma dilution. In the fat pad assay, it would appear that something other than insulin alone is being measured in view of the high insulin-like activity in fasting plasma and its persistence after pancreatectomy; for example, epinephrine has been shown to increase glucose-1-C^{14} oxidation although its effect on oxidation of C-6 is much greater [*49*].

Clearly, the most rewarding information is likely to be derived from some combination of the specific immunoassay technic with one or another of the bioassay methods wherein it may be possible to disentangle the effects of insulin from those of other plasma substances (as yet unidentified but possibly important) influencing glucose metabolism.

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* Antoniades [*47*] has reported that insulin-like activity is lost from plasma collected through certain cationic exchange resins and can be recovered from the resins by elution. Since exogenous insulin, added *in vitro*, is not adsorbed by the resin, Antoniades and Gundersen [*14*] have suggested that the bulk of endogenous plasma insulin is bound to a basic plasma protein (which is responsible for its adsorption by resin) and that in this form it is unable to act on the diaphragm but exhibits full hormonal activity in the fat pad. However, this hypothesis remains to be established. The studies of Moloney and Coval [*48*] have suggested that there may be *immunologic* differences between endogenous and extracted insulin in some species.

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Clinical Studies

Hereditary Persistence of Fetal Hemoglobin*

A Family Study

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SICKLE cell anemia (homozygous hemoglobin S disease) is a disorder characterized by unrelenting hemolytic anemia, chronic jaundice and recurring episodes of pain in the abdomen and extremities. Several persons have been encountered whose red cells appeared to contain only hemoglobin S on filter paper electrophoresis, but who did not present anemia or the typical manifestations of sickle cell disease. In these instances further investigation revealed an increased concentration of fetal hemoglobin in the red cell hemolysates. Family studies excluded the possibility that the persons under observation were homozygous for the gene determining hemoglobin S and provided clear evidence that a previously unrecognized inherited abnormality of the red cells was involved [*1–3*]. The result of this genetically determined anomaly was a persistence of fetal hemoglobin in high concentration in the erythrocytes, not accompanied by hematologic or clinical manifestations. When this abnormality occurred in persons who were also heterozygous for hemoglobin S, only hemoglobins S and F were demonstrable, yet there was little or no anemia or other evidence of disease. The purpose of this report is to describe clinical, hematologic and genetic studies of members of three generations of a family in which hereditary persistence of fetal hemoglobin was found in conjunction with hemoglobins A or S.

METHODS

The volume of packed red cells was determined by a micro-hematocrit technic or by the method of Wintrobe [*4*]. Hemoglobin was measured as oxyhemoglobin in a Beckman spectrophotometer at 544 mμ. The instrument had been calibrated using a cyanmethemoglobin standard [*5*]. Tests for sickling of erythrocytes were performed using sodium metabisulfite as a reducing agent [*6*]. The red cells were counted with an electronic counter.‡ Fetal hemoglobin (i.e., the fraction resistant to denaturation by alkali) was measured by the method of Singer and his associates [*7*]. Hemolysates of saline-washed erythrocytes were subjected to electrophoresis on filter paper using the method of Smith and Conley [*8*], and on agar gels at pH 6 by the method of Marder and Conley [*9*]. Osmotic fragility of the red cells was measured by the technic described by Emerson and his associates [*10*]. Serum haptoglobin concentration was estimated by the method of Herman [*11*].

CLINICAL AND EXPERIMENTAL STUDIES

Electrophoresis of hemoglobin on filter paper is regularly performed on the blood of Negro patients in The Johns Hopkins Hospital. In the case to be described the electrophoretic pattern was similar to that of sickle cell anemia, although the patient had no manifestations of that disease.

J. M. G. (JHH No. 795700), a twenty-three year old Negro woman, complained that she had had pain in her chest and mild shortness of breath on exertion for two years. There was no family history of anemia or other relevant disorders. The past health of the patient had been generally good and she had not had anemia, jaundice, abdominal pain or other symptoms usually encountered in patients with sickle cell anemia. She had been emotionally unstable and had experienced short episodes of uncontrollable tantrums, each associated with excessive intake of alcohol. These tantrums were followed by periods of unconsciousness, but there was no history of convulsive movements. She had had two uncomplicated pregnancies.

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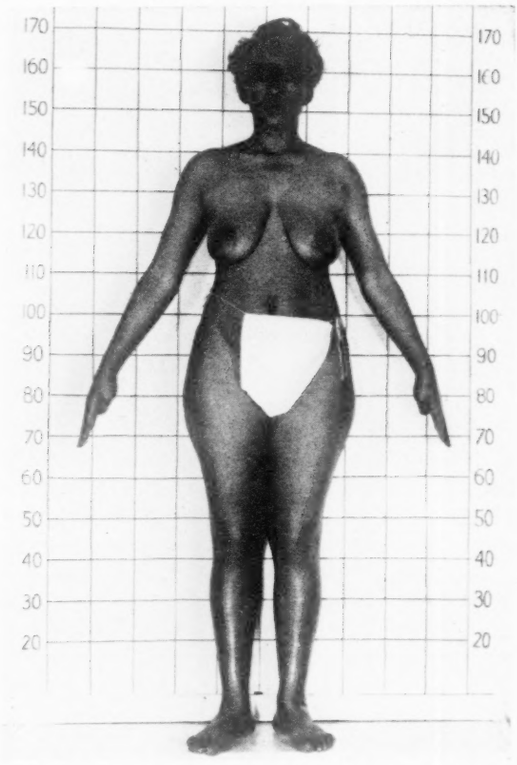


FIG. 1. The propositus, J. M. G. Body proportions are within normal limits and the patient does not display the abnormal habitus which is characteristically seen in patients with sickle cell anemia.

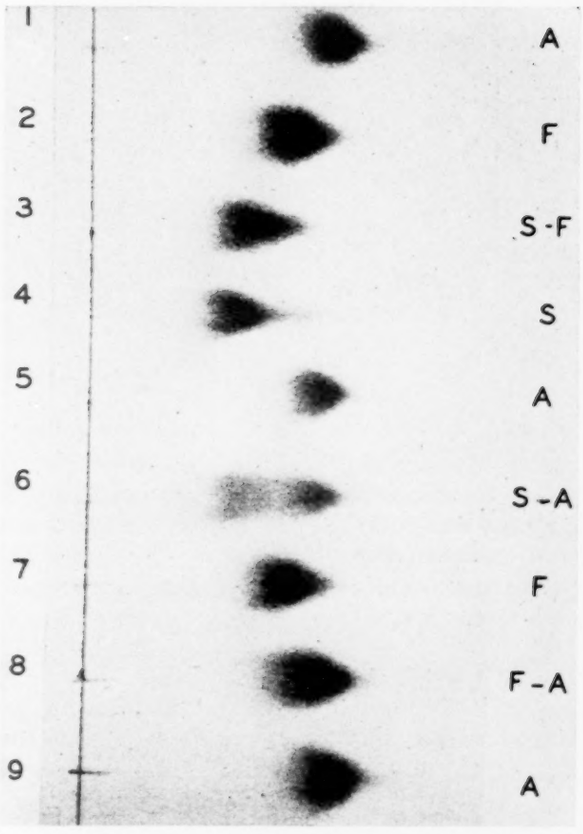


FIG. 2. Electrophoretic patterns on filter paper (veronal buffer, pH 8.6) of hemoglobin obtained from (1) normal adult, (2) normal umbilical cord, (3) the propositus, J. M. G., (4) patient with sickle cell anemia, (5) normal adult, (6) person with sickle cell trait, (7) normal umbilical cord, (8) child of the propositus, (9) normal adult.

On physical examination the patient was a healthy-appearing, well developed, dark-skinned young Negro woman, rather childish in affect and of limited intelligence. The stature and habitus were within normal limits. (Fig. 1.) There was no pallor or jaundice, and the integument was not unusual. The head and facial structures were normal except for carious teeth. The heart and lungs were normal and the liver and spleen could not be palpated. The left extremities were somewhat smaller than the right and showed slight weakness. There were no pathological reflexes.

The hematocrit value was 43 per cent, red cell count 5.1 million per cu. mm., hemoglobin 14 gm. per cent, and white cell count 6,000 per cu. mm. with 58 per cent neutrophils, 2 per cent eosinophils, 34 per cent lymphocytes and 6 per cent monocytes. Platelets were normal in number and form. The red cells were normochromic with slight anisocytosis, and rare target cells were seen. Sickling occurred promptly in preparations of fresh blood mixed with sodium metabisulfite. The reticulocyte count was 1 per cent and the icterus index was 5 Meulengracht units. Haptoglobin was sufficient to bind more than 100. mg. hemoglobin per 100 ml. of serum, a normal value. Urinalysis, roentgenograms of the chest and skull, and an electroencephalogram were within normal limits. The electrocardiogram showed minor and questionably significant T wave abnormalities.

An organic cause of the patient's complaints could not be demonstrated. The only significant abnormality apart from the hemoglobin disorder appeared to be slight evidence of old hemiparesis.

The discovery of an electrophoretic pattern of hemoglobin characteristic of sickle cell anemia in a patient who displayed neither anemia nor symptoms of sickle cell disease led to further studies of the patient and of her family.

Demonstration of Elevated Fetal Hemoglobin Concentration in Hemolysates of the Patient's Blood. The electrophoretic pattern of the patient's hemoglobin on filter paper in veronal buffer at pH 8.6 (Fig. 2) indicated that there was a small component, in the position of fetal hemoglobin, slightly anodal to the major S component. When the hemoglobin was subjected to electrophoresis on filter paper in 0.05 M phosphate buffer at pH

TABLE 1*

DATA PERTAINING TO MEMBERS OF THE FAMILY SHOWN IN THE PEDIGREE IN FIGURE 3

Family Member	Age (yr.) and Sex	Hemoglobin Components on Electrophoresis		Alkali-Resistant Hemoglobin (%)	Hematologic Values				Erythrocyte Morphology					
		Paper (pH 8.6)	Agar (pH 6.0)		Hematocrit (%)	Red Cell Count (millions/cu. mm.)	Hemoglobin (gm. %)	Reticulocytes (%)	Sickling	Anisocytosis	Poikilocytosis	Stippling	Hypochromia	Target Cells
I-1	42, M	A(F)*	AF	28	44	4.67	13.3	1.0	Negative	++	+	0	0	+
I-2	41, F	AS	AS(F)	1.1	32.8	4.32	8.7	1.4	Positive	+	+	0	+	+
II-1	24, F	S(F)	SF	28	43	5.13	13.8	1.0	Positive	+	±	0	+	+
II-4	16, F	S(F)	SF	29	34	4.41	10.8	4.1	Positive	+	+	0	+	+
II-7	12, F	S(F)	SF	27	39	5.44	12.7	...	Positive	+	±	0	0	+
II-2	20, M	AS	AS(F)	0.6	47	4.94	14.2	...	Positive	0	0	0	0	0
II-3	18, M	AS	AS(F)	0.3	44.5	5.61	13.9	...	Positive	+	0	0	+	0
II-5	14, F	AS	AS(F)	0.9	40.5	5.18	13.4	...	Positive	±	0	0	0	0
II-6	13, F	AS	AS(F)	2.2	38	5.94	11.4	...	Positive	+	++	0	0	+
II-8	12, F	AS	AS(F)	0.5	39	5.02	12.1	...	Positive	++	0	0	0	0
III-1	8, M	A(F)	AF	26	39	4.89	12.6	...	Negative	±	±	0	0	+
III-2	6, F	A(F)	AF	24	37.5	5.35	11.9	0.4	Negative	±	0	0	+	0
III-3	1, F	AS(F)	.....	....	31	....	....	...	Positive	+	+	0	0	+
III-4	2, F	S	.....	15	30	3.91	8.3	...	Positive	++	+	0	+	+
III-5	1/12, F	.....	.....	....	26	....	....	...	Negative	+++	++	0	++	++

* The inclusion of F in parens (F) denotes that fetal hemoglobin was hardly detectable by the method employed or that its concentration did not appear to exceed that in normal blood. The symbol ± indicates a questionably significant deviation from normal; + indicates rare or slight abnormality, ++ moderate and +++ marked abnormality.

6.5, a small component migrated toward the cathode more slowly than the S fraction. This minor component was larger than that seen in patterns from most patients with sickle cell anemia, and had the mobility of hemoglobin F. On electrophoresis in agar gel in citrate buffer at pH 6, the patient's hemoglobin showed hemoglobin S and a prominent spot with the configuration and mobility of fetal hemoglobin. The presence of fetal hemoglobin in increased concentration was confirmed by the demonstration that the alkali-resistant fraction constituted 28 per cent of the total hemoglobin.

Demonstration of the Apparent Absence of Hemoglobin A from Hemolysates of the Patient's Blood. Hemoglobin A could not be detected in the filter paper patterns of the patient's hemolysate. In an artificial mixture of hemoglobins S and A, a component of hemoglobin A amounting to as much as 10 per cent of the total could not be detected by use of paper electrophoresis [*12*]; accordingly, additional studies were performed to determine whether hemoglobin A might be present in low concentration. The following artificial mixtures were prepared using umbilical cord blood containing 88 per cent alkali-resistant hemoglobin, hemoglobin from a normal adult containing 0.3 per cent alkali-resistant hemoglobin, and hemoglobin from a patient with sickle cell anemia containing 3 per cent alkali-resistant hemoglobin:

Hemoglobin (%)		
F	S	A
25	63	12
25	69	6
25	72	3

Aliquots of these mixtures were subjected to electrophoresis on agar gel in citrate buffer and compared to the separations obtained by the same method employing hemolysates of the patient and of her sister (II-7) who had the same hemoglobinopathy. Hemoglobin A could not be detected in blood from these two individuals, although it was easily recognized in the artificial mixture in which it was present in a concentration of only 3 per cent.

Family Study. Both parents, seven siblings, two children and three nieces of the patient

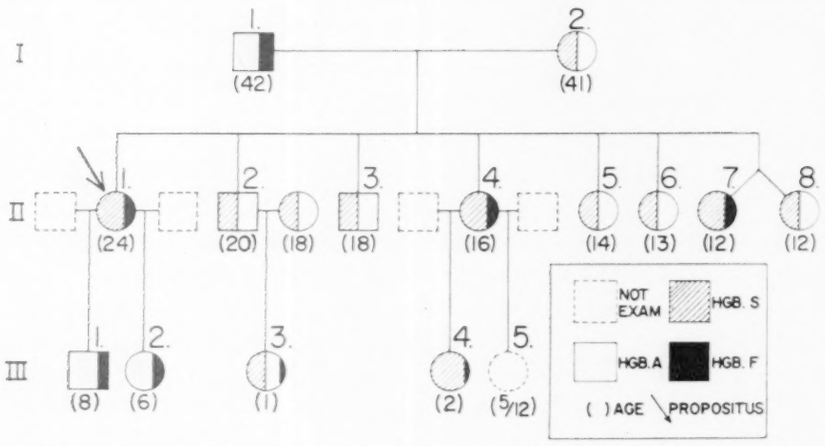


FIG. 3. Pedigree of the family of the patient, J. M. G., in which hemoglobins are shown as phenotype.

were available for study. The family pedigree is shown in Figure 3, and hematologic values are summarized in Table I.

The mother of the propositus showed the electrophoretic pattern of sickle cell trait and her red cells could be made to sickle. There was a normal amount of fetal hemoglobin in her blood. The father appeared to be normal clinically and hematologically. The electrophoretic pattern of his hemoglobin on filter paper showed hemoglobin A, with a trail in the position of fetal hemoglobin. Electrophoresis of his hemoglobin on agar showed increased fetal hemoglobin, and the alkali-resistant fraction constituted 28 per cent. Results of ABO, Rh and MN typing were compatible with paternity of the generation II offspring.

In the second generation three persons including the propositus showed hemoglobins S and F (S-F) on electrophoresis and in none of these was hemoglobin A detected. In each the fetal hemoglobin was strikingly increased above normal as measured by alkali denaturation. One who was seven months pregnant had a hematocrit value of 32 per cent, a value not unusual in this clinic among normal pregnant women, and the two others were not anemic. The remaining four persons in this generation had the sickle cell trait, and in these there was no elevation of the concentration of fetal hemoglobin. Each member of generation II had inherited from the mother the gene for hemoglobin S, an occurrence expected in only one of 256 chances.

In the third generation two children of the propositus showed no hemoglobin S in their hemolysates, but both had elevated levels of fetal hemoglobin. These children appeared healthy. There was no jaundice, hepatic or splenic enlargement or other abnormality. Careful examination of their blood smears revealed a slight and equivocally significant degree of anisocytosis. The abnormalities characteristically seen in the blood of persons with thalassemia minor were not present. Fresh and incubated blood of both children showed no abnormal resistance to osmotic hemolysis.

Through the courtesy of Dr. Park S. Gerald measurements of hemoglobin A_2 were made on hemolysates prepared from the blood of the propositus and her two children with the A-F pattern. Hemoglobin A_2 comprised 2.1 per cent of the total hemoglobin of the mother (J. M. G., II-1) and 2.1 and 2.4 per cent, respectively, of the total hemoglobin of the children (III-1 and III-2). Even when calculated as per cent of the non-fetal fraction, these values do not exceed the normal (3.1 per cent or less).

Studies of the other children in generation III were incomplete. The child designated as III-3, aged one year, had hemoglobins S and A and apparently a small amount of hemoglobin F on paper electrophoresis. Child III-4, aged two, appeared to have only hemoglobin S on paper electrophoresis. Her blood sickled readily in sodium metabisulfite solution. She had 15 per cent alkali-resistant hemoglobin, a high level at this age.

COMMENTS

Electrophoresis of hemoglobin on filter paper is a simple and invaluable method for differ-

entiating sickle cell anemia from other disorders in which sickling of the red cells occurs. Nevertheless, it has become apparent that the method has definite limitations. The sickling phenomenon and an electrophoretic pattern indistinguishable from that of sickle cell anemia may be encountered in four different inherited disorders of the red cell: (1) In sickle cell anemia the patient is homozygous for the gene giving rise to the hemoglobin abnormality so that hemoglobin S appears as virtually the only component [*13*]. (2) In sickle cell hemoglobin D disease the electrophoretic pattern of hemoglobin is identical with that of sickle cell anemia because hemoglobins S and D have the same electrophoretic mobility [*14*]. (3) In some instances of sickle cell thalassemia disease, hemoglobin A is not detected by electrophoresis, presumably because of suppression of its formation by the thalassemia gene, and the electrophoretic pattern cannot be distinguished from that of sickle cell anemia [*15*]. (4) In cases such as that described in this report, simultaneous occurrence of sickling and inherited persistence of fetal hemoglobin is associated with an electrophoretic pattern similar to that of sickle cell anemia.

Additional studies are required to establish a precise diagnosis of these conditions; particularly when the clinical features of the disease are atypical for sickle cell anemia, such definitive studies are indicated. Electrophoresis of hemoglobin on agar gels at pH 6 has been shown to separate hemoglobins S and D [*16*] and provides an unequivocal means for differentiating sickle cell hemoglobin D disease from the other sickling disorders [*17*]. For specific diagnosis of the three remaining conditions there is at present no substitute for examination of appropriate members of the family of the patient.

Edington and Lehmann [*1*] in 1955 drew attention to the situation in which only hemoglobins S and F were found in individuals who did not have the usual clinical features of sickle cell anemia. They presented genetic evidence that this was not a homozygous hemoglobin S disorder despite the high proportion of hemoglobin S in the hemolysate. They described two adult males with normal hematocrit values, paper electrophoretic patterns showing only hemoglobin S, but with fetal hemoglobin concentrations which were elevated as measured by the alkali denaturation technic. Each of these subjects had a child whose red cells contained no hemoglobin S, thereby demonstrating that the parent was not homozygous for the sickling gene.

Griggs and Harris [*19*] encountered two sisters, each over the age of fifty, with no history of anemia or symptoms of sickle cell disease but with 70 per cent hemoglobin S and 30 per cent fetal hemoglobin in their red cell hemolysates. One sister was in the terminal stages of uremia and was severely anemic, but the other, who was in good health, had a hemoglobin concentration of 13.5 gm. per cent.

Neel and his associates [*18*] also found two asymptomatic and non-anemic persons whose red cells contained only hemoglobins S and F, but family studies were not reported. These investigators observed several hematologically unaffected individuals in whose blood the presence of elevated levels of fetal hemoglobin was associated with hemoglobin A.

Jacob and Raper [*2*] reported four cases in which only hemoglobins S and F were demonstrable, yet the characteristic manifestations of sickle cell anemia were lacking. Each of two family studies showed that one parent had sickle cell trait and the other had hemoglobins F and A; thus the propositi could not have been homozygous for hemoglobin S.

Went and MacIver [*3*] studied three generations of a Jamaican family in which this disorder was encountered. In the index case, only hemoglobin S was demonstrable on filter paper electrophoresis although the father was found to have sickle cell trait and the mother only hemoglobins A and F. The genetic contribution of the patient to her children could not be evaluated because the spouse had sickle cell trait. However, two children had hemoglobins S and F.

In the family described in the present report, the data are consistent with the hypothesis that the genetic condition resulting in persistence of fetal hemoglobin is allelomorphic with the genes responsible for hemoglobins A, S and C, at a locus now usually designated Hb $^{\mathrm{ASC}}_{1}$ [*20*]. The chemical characteristics of the hemoglobin produced as a result of this genetic anomaly, as far as they have been studied, are the same as those of the fetal hemoglobin of cord blood. There is good evidence that production of fetal hemoglobin in normal persons is not determined by a gene allelic with that for hemoglobin A. In persons with the disorder under consideration, it is not known whether the production of fetal hemoglobin is the direct result of gene action.

TABLE II

HEMATOLOGIC AND FAMILY DATA IN REPORTED CASES WITH S AND F HEMOGLOBINS

Case No.	Age (yr.) and Sex	Alkali-Resistant Hemoglobin (%)	Hematocrit (%)	Hemoglobin (gm. %)	Reticulocytes (%)	Hemoglobin Type of			
						Father	Mother	Siblings	Children
Jacob, G. F. and Raper, A. B. [2]									
1	18, M	15	49.5	14.7	.....	AS	AF	AS	.....
2	23, M	22	47	15.0	<0.5	AF	AS	4 AS A	.....
3	20, F	20	....	13.3	<1	...	...	.....	.....
4	24, M	10	49	16.4	<1	...	...	.....	.....
Edington, G. M. and Lehmann, H. [1]									
5	40, M	5.9	....	11.9	1.2	...	...	.....	AC AF
6	30, M	26	....	14.5	0.6	...	...	.....	AF
Neel, J. V. et al. [18]									
7	28, M	13.2	....	11.5	.....	...	...	.....	.....
8	Adult, M	6.6	....	11.5	.....	...	...	.....	.....
Griggs, R. C. and Harris, J. W. [19]									
9	51, F	30	....	13.5	.....	...	...	SF	.....
Went, L. N. and MacIver, J. E. [3]									
10	26, F	27	38	12.5	3	AS	AF	3 AS A, AF SF	AF, AS SF, SS SAF*
11	16, M	24	32	10.2	4	AS	AF		.....
12	1, M	12	39	9.1	6	AS	SF	AF SS AS SAF*	.....
Herman E. C., Jr. and Conley, C. L. (Present Series)									
13	24, F	28	43	13.8	1.0	AF	AS	2 SF 5 AS	2 AF
14	16, F	29	32	10.8	4.1	"	"		S
15	12, F	27	39	12.7	.....	"	"		.....

* Infant.

More likely fetal hemoglobin is produced as the indirect result of failure of formation of hemoglobin A. The genetic condition could be accounted for by deletion of a gene at the ASC locus. It is clear that the genetic abnormality, when occurring in conjunction with a gene for hemoglobin A, does not inhibit production of normal hemoglobin. There are as yet no reports of the occurrence of this genetic anomaly together with hemoglobins other than A or S, although Oleson and associates [*21*] consider the possibility that they may have encountered it in conjunction with the thalassemia gene. It would be most helpful to know conclusively of the proof

TABLE III

HEMATOLOGIC DATA IN REPORTED CASES WITH A AND F HEMOGLOBINS

Case No.	Age (yr.) and Sex	Alkali-Resistant Hemoglobin (%)	Hematocrit (%)	Hemoglobin (gm. %)	Red Cell Count (million per cu. mm.)	Reticulocytes (%)	Osmotic Fragility (NaCl %)	Erythrocyte Morphology					
								Anisocytosis	Poikilocytosis	Stippling	Polychromatophilia	Target Cells	Hypochromia
Jacob, G. F. and Raper, A. B. [2]													
1	45–50, F	22	46	12.6	5.1	...	.42–.34	+	0	..	..	0	..
2	Adult, M	12	43.5	13.1	5.3	...	.42–.30	0	0	..	..	±	..
Edington, G. M. and Lehmann, H. [1]													
3	Adult, F	5	....	12.2	....	2.4	50% at .50	....	..	..	..	0	..
4	4½, M	24	....	9.6	....	1.0	50% at .37	....	..	..	..	±	..
Went, L. N. and MacIver, J. E. [3]													
5	53, F	24	45	15.0	5.0	1.0	normal	....	..	..	..	±	+
6	14, M	26	43	14.0	5.0	0.5	normal	....	..	..	..	0	0
Herman, E. C., Jr. and Conley, C. L. (Present Series)													
7	42, M	28	44	13.3	4.67	1.0	.....	++	+	0	+	±	0
8	8, M	26	39	12.6	4.89	...	.55–.25	±	±	0	0	±	0
9	6, F	24	37.5	....	....	...	.45–.25	±	0	0	0	0	±

of paternity of the son of patient J. A. S. in the report of Edington and Lehmann [*1*], for here a person with hemoglobins A and C was apparently the child of a parent with hemoglobin S-F. No case has been reported in which the genetic state responsible for persistence of fetal hemoglobin was present in homozygous form. What the phenotype of such an individual would be is conjectural. One might expect only hemoglobin F to be present. The clinical severity of such a hemoglobinopathy is also open to speculation.

Data from fifteen cases in which persistence of fetal hemoglobin occurred with hemoglobin S are summarized in Table II. All the patients were Negroes. Except for one infant with hypochromic anemia, all had hemoglobin concentrations above 10 gm., a level not attained by many patients with sickle cell anemia. Icterus was noted only once, and smears of the blood were not strikingly abnormal. Episodes suggestive of hemolytic crises have not been encountered. In our case the normal icterus index, reticulocyte count and haptoglobin level indicate that there was not a compensated hemolytic disorder. The subjects have not been described as having an abnormal habitus and have been generally quite well. Nevertheless, some have had manifestations which may have been related to the sickling disorder, including aseptic necrosis of the femoral heads (two subjects), joint pains (one subject), and in our case evidence of mild hemiparesis.

Although the data are scanty, there is no suggestion that the persistence of fetal hemoglobin in association with hemoglobin A leads to hematologic abnormality or clinical illness. Results of hematologic examinations of nine persons with this disorder are summarized in Table III.

The genetic abnormality causing persistence of fetal hemoglobin is clearly different from the thalassemia gene. Persons with thalassemia minor usually display characteristic morphologic abnormalities of the red cells including hypochromia, stippling and target forms; fetal hemoglobin is usually normal or only slightly increased in concentration, but the hemoglobin A_2 fraction is larger than in normal blood. The differences between the manifestations of thalassemia minor and persistence of fetal hemoglobin are summarized in Table IV.

The clinical manifestations of sickle cell anemia are thought to be wholly the result of the abnormal hemoglobin in the red cells [*13*]. *In vitro* studies have demonstrated a relationship between the sickling phenomenon and the concentration of hemoglobin S in the red cell hemolysate [*22*]. A correlation has been noted

TABLE IV

COMPARISON BETWEEN THALASSEMIA MINOR AND HEREDITARY PERSISTENCE OF FETAL HEMOGLOBIN

	Physical Abnormalities	Hemoglobin Concentration	Red Cell Morphology	Osmotic Fragility of Red Cells	Hemoglobin A_2	Fetal Hemoglobin
Thalassemia minor	Sometimes slight pallor, jaundice, splenomegaly	Reduced	Hypochromia, target cells, stippling	Reduced	Usually increased	Normal or slightly increased
Hereditary persistence of fetal hemoglobin	None known	Normal	Virtually normal	Normal	Normal	Increased

between the concentration of hemoglobin S and the severity of clinical symptoms [*19*], although Singer and his associates [*23*] have pointed out the occurrence of outstanding exceptions to this generalization. Cases such as that described here emphasize that a high concentration of hemoglobin S in the erythrocytes and pronounced sickling of the red cells *in vitro* may be unaccompanied by hemolytic anemia. An explanation for the benign nature of this disorder is provided by the observations made by Griggs and Harris [*19*]. These investigators studied the effects of deoxygenation on the viscosity of whole blood from patients with various sickle disorders. Red cells from their patient with the S-F pattern responded to anoxia more like the cells of sickle cell trait than those of sickle cell anemia, even though 70 per cent of the hemoglobin was identified as S. A mechanism by which the cells are apparently protected from destruction is suggested by previous studies. Watson and her colleagues [*24,25*] have shown that the erythrocytes of newborns are protected from sickling by the high concentration of fetal hemoglobin, and that the percentage of cells which can be made to sickle is inversely related to the concentration of hemoglobin F. Allison [*26*] demonstrated that fetal hemoglobin does not appear to enter into the formation of molecular aggregates with sickle hemoglobin, in contrast to the behavior of hemoglobins A and C. However, Singer and his associates [*7*] found no correlation between concentration of fetal hemoglobin and severity of anemia in patients with sickle cell anemia. Some patients with apparently classic sickle cell anemia have been reported to have concentrations of fetal hemoglobin in excess of 20 per cent, as measured by electrophoresis [*15*] and by the alkali denaturation technic [*7*]. If the latter observations are correct, the possibility must be considered that the fetal hemoglobin in the cases under discussion differs in some way from that occurring in the blood of patients with sickle cell anemia.

At present almost nothing is known concerning the frequency and distribution of the inherited disorder which results in persistence of fetal hemoglobin. Because hemoglobins A and F are not well differentiated by filter paper electrophoresis, persons with the A-F pattern have undoubtedly been overlooked in the numerous surveys which have been performed. Electrophoresis of hemoglobin in an agar medium at low pH should be particularly useful in surveys to determine the incidence of this anomaly.

SUMMARY

An abnormality of the red cells manifested by persistence of fetal hemoglobin was traced through three generations of a Negro family. This anomaly appears to be genetically determined by a factor allelic with the genes for hemoglobins A, S and C. The primary effect of this genetic disorder is unknown, but quite possibly is a failure of production of hemoglobin A, with formation of fetal hemoglobin occurring as an indirect result.

In members of the family who showed persistence of fetal hemoglobin together with hemoglobin A, there were no clinical or hematologic abnormalities other than the presence of fetal hemoglobin in high concentration.

Three members of the family had inherited the gene for hemoglobin S in addition to that resulting in persistence of fetal hemoglobin. These persons appeared to be well and had none of the manifestations of sickle cell anemia. Nevertheless their red cells contained predominantly hemoglobin S, and hemoglobin A could not be

detected in their red cell hemolysates. The benign nature of this disorder is probably attributable to the presence of fetal hemoglobin in high concentration in the red cells and the resultant resistance to sickling.

Acknowledgment: Valuable technical assistance was provided by Miss Anne Bjerklie, Mrs. Helen French and Mrs. Margaret Causton. Blood typings were performed in the Blood Bank through the courtesy of Miss Sarah Stallings. We are indebted to Dr. Park S. Gerald, The Children's Medical Center, Boston, Massachusetts, for the quantitative measurements of hemoglobin A_2.

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Hemoglobin Metabolism in Thalassemia*

In Vivo Studies

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THE anemia in thalassemia major is in part hemolytic, caused by an intraerythrocytic defect, and in part due to relative failure of the bone marrow, when ineffective erythropoiesis prevents adequate compensation for the shortened cell survival. In addition, hemoglobin formation may be abnormal, since hypochromia of the erythrocytes occurs in the presence of an abundance of iron. Previous *in vitro* investigations have in fact, provided evidence of such an abnormality and suggested that it might be due to a defect in heme synthesis [*1*]. The present work was undertaken to investigate *in vivo* the apparent disturbance of hemoglobin formation. Glycine-2-C^{14} was administered orally to patients with thalassemia major; the subsequent tagging of heme, globin and stercobilin was measured. The results demonstrated poor apparent utilization of glycine for hemoglobin synthesis, relatively less tagging of heme than of globin so that the ratio of heme to globin radioactivity was low, and remarkably high tagging of the stercobilin formed during the first few days after administration of glycine-2-C^{14}. The shortened red cell survival in thalassemia major also was confirmed. Values obtained from control observations on normal subjects were used for comparison.

Shemin and his collaborators first demonstrated that glycine is specifically utilized in the biosynthesis of heme [*2,3*]. Subsequent studies by these and other workers have filled in many of the steps in the biosynthetic pathway of hemoglobin; detailed references may be found in recent reviews [*5,6*]. The *alpha* carbon atom [*7,8*], but not the carboxyl carbon atom [*9–11*] of glycine is utilized in heme formation, whereas the whole molecule is incorporated into globin as one of its constituent amino acids. After administration of N^{15}- or C^{14}-tagged glycine the disappearance of the labeled hemoglobin from the peripheral blood has been used as a measure of red cell life span in animals and in man, in normal subjects, and in patients with various red cell disturbances [*4,10,12–16*]. In addition, the comparative labeling of heme and of globin has been investigated in the rat and rabbit [*17,18*].

Measurement of the isotope content of stercobilin after administration of N^{15}-glycine has confirmed that, in the normal subject, much of the excreted stercobilin is derived from the breakdown of hemoglobin at the end of the life span of circulating red cells [*19,20*]. An appreciable fraction, however, in which the isotope appears in high concentration during the first few days after administration, seems to be derived from some other source. The origin of this early labeled stercobilin remains a mystery. It has been suggested that it might be derived (1) from hemoglobin liberated in the marrow from nucleated red blood cells, (2) from the hemoglobin of red corpuscles which are destroyed soon after formation either in the marrow or shortly after delivery to the circulation, (3) from heme formed in excess of globin, or (4) by direct synthesis from a pyrrol compound without intermediate heme formation. The view that it is a by-product of hemopoiesis receives support from the recent demonstration by Gray and Scott [*21*] that, after administration of C^{14}-

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glycine the radioactivity of the early labeled stercobilin was greatly increased when hemopoiesis was stimulated by hemorrhage. A somewhat similar result was obtained in an earlier investigation using N^{15}-glycine, but a different interpretation was offered [*23*]. The proportion of early labeling has been found to be considerably greater than the normal in pernicious anemia [*23*] and congenital porphyria [*20,24,25*].

It was of interest to study the early-labeled stercobilin in thalassemia, particularly since Sturgeon and Finch [*26*] have pointed out that the urobilinogen output in this disease is greater than can be explained satisfactorily by the apparent rate of breakdown of circulating hemoglobin. The same phenomenon was observed in pernicious anemia [*27*]. These authors suggest that there may be considerable *ineffective erythropoiesis*, so that the excess urobilinogen might be derived from premature destruction of red cells in the marrow. Our study of one patient with thalassemia who was fed glycine-2-C^{14} demonstrated remarkably high radioactivity in the early labeled stercobilin; this result is not inconsistent with Sturgeon and Finch's suggestion, but it is also compatible with any one of the four possible explanations tabulated.

MATERIALS AND METHODS

Subjects. Preliminary investigations were made on a dog rendered anemic by repeated bleeding, and given glycine-2-C^{14} by injection. Some data from this experiment are included subsequently for comparison. Two adult males, with no evidence of any hematologic disorder, served as normal control subjects. Three children suffering from thalassemia major were studied; the main hematologic data on these patients and their parents are shown in Table I; their clinical histories are summarized in the appendix.

Tagging of hemoglobin was investigated in all five subjects; stercobilin tagging was measured in Case II only, during a second investigation on this patient. The children were ambulant, indeed attending school regularly, except that one (Case II) was in the hospital in cardiac failure during the second period.

Administration and Dose. Since these patients were receiving blood transfusions at short intervals, it was necessary to sandwich the investigations into the period between transfusions. The depressant effect of blood transfusion on hemopoiesis in thalassemia has been demonstrated by Smith and his colleagues [*33*]. Although erythropoietic depression may last for at least two weeks, it should be diminishing after about ten days. The oral dose of glycine was therefore administered ten to fourteen days after a transfusion. This plan allowed an adequate period for study in Cases I and II (forty-six and fifty-two days respectively), but too short a period for complete study in Case III. In the second investigation in Case II, glycine-2-C^{14} was administered five days after a transfusion, and a further transfusion was required on the twenty-third day, but the investigation was continued and an appropriate correction applied for the dilution (*vid. seq.*). A tracer dose of approximately 100 μc. of glycine-2-C^{14} was administered to each subject.* It was dissolved in 100 ml. of water and given orally in nine doses over three days, except in the second investigation of Case II, when it was given over thirty-six hours. The actual doses administered are shown in Table II.

Isolation and Counting Procedures. Venous blood samples were drawn at intervals of three to nine days from the patients with thalassemia, and two to seventy days from the normal subjects. A minimum of 10 ml. of blood was required for the preparation of protoporphyrin and globin.

Methods used for the isolation of protoporphyrin and globin, and for the measurement of C^{14} radioactivity have been described elsewhere [*1*], and are only briefly outlined here. Protoporphyrin was isolated from hemoglobin as the crystalline methyl ester [*37*], after the free protoporphyrin present in red cells had first been completely removed. Globin was prepared by a modification of the Anson and Mirsky procedure [*38*].

During the stercobilin investigation complete stool collections were made. Single twenty-four-hour collections were analyzed separately for the first sixteen days; thereafter aliquots of three-day stool collections were analyzed. Stercobilin was isolated by a modification of Watson's method [*39,40*]. The total stool urobilinogen was determined in an aliquot of the ferrous hydroxide mixture after centrifugation and filtering. Stercobilin was isolated from the rest of the filtrate and finally crystallized from solution in chloroform, using hot acetone. Yields were small, averaging less than 10 per cent of the theoretical yield.

Radioactivity was measured in a thin window, gas flow counter (Nuclear Chicago, Model D 47); a minimum of 4,000 counts was recorded from each sample. Protoporphyrin and stercobilin were counted as thin layers, and globin as a thick layer. Correction for self-absorption was made from experimentally determined correction curves for protoporphyrin and globin. The samples of stercobilin used for counting were extremely small, so that no correction for self-absorption was necessary. Radioactivity was expressed

* Much of the C^{14} leaves the body as respiratory $C^{14}O_2$ during the first few days after administration [*34*], and the proportion of the dose retained in the tissues is small [*35*]. One hundred microcuries of C^{14} has been regarded as a maximum permissible dose in adults [*36*]. The use of a 100 μc. dose was considered justifiable in this investigation in view of the patients' relatively short life expectancy.

TABLE I

HEMATOLOGIC DATA

Subject, Age (yr.)	Hemoglobin (gm./100 ml.)	Red Blood Cells (million/cu. mm.)	Hematocrit (%)	Mean Cell (volume cu. μ)	Mean Corpuscular Hemoglobin Concentration (%)	Immature Red Cells (%)		Hemoglobin Type (%) Fetal (alkali denaturation)	Serum Iron (μg./100 ml.)	Unsaturated Iron-Binding Capacity of Serum (μg./100 ml.)	Free Erythrocyte Protoporphyrin (μg./100 ml. cells)	Diagnosis and Remarks
						Reticulocytes	Normoblasts					
A. S. (propositus [*41*])	5.2	2.08	18	87	28	4–6	2–6	A 19% F	224	0	43	Thalassemia major (splenectomy)
G. S. (father [*43*])	11.8	5.94	40	68	30	...		A 1.2% F	111	216	96	Thalassemia trait
M. S. (mother [*41*])	11.7	5.53	40	72	29	...		A 2.1% F	90	90	74	Thalassemia trait
A. L. (propositus [*10*])	4.6	1.56	16	102	29	2–7	0.5–3	A 28% F	175	0	64	Thalassemia major (splenectomy)
M. L. (father [*40*])	15.2	6.89	50.5	73	30	2		A 1.9% F	222	153	...	Thalassemia trait
L. L. (mother [*37*])	11.3	5.35	38	69	30	2.7		A 2.2% F	135	189	...	Thalassemia trait
D. G. (propositus [*8*])	5.0	2.20	17	77	29	0.1		A 2.8% F	138	0	86	Thalassemia major
J. G. (father [*41*])	14.9	5.91	49	83	30	...		A 1.6% F	123	135	...	Thalassemia trait
A. G. (mother [*32*])	11.7	5.28	28	72	31	...		A 2.4% F	74	225	...	Thalassemia trait

NOTE: Data on the propositi are representative values before transfusion (at the time of the first investigation on A. L.). References to some of the methods used are as follows: Filter paper electrophoresis of hemoglobin [*28*]; determination of fetal hemoglobin by alkali denaturation [*29*]; serum iron [*31*]; unsaturated iron-binding capacity of serum [*31*]; and free erythrocyte protoporphyrin [*32*].

TABLE II

INCORPORATION OF GLYCINE-2-C^{14} INTO CIRCULATING HEMOGLOBIN

Subject Age (yr.), Diagnosis	Dose (μc. and ct./min.)	Body Weight (kg.)	Day of Study	Calculated Blood Volume* (ml.)	Observed Specific Activities† (ct./min./mg.)		Calculated Specific Activity of Hemoglobin (ct./min./gm.)	Hemoglobin Level (gm./100 ml.)	Total Circulating Hemoglobin (gm.)	Total Maximum Incorporation (ct./min.)	Per cent of Dose Incorporated
					Protoporphyrin	Globin					
S. H., normal	124 93 × 10^6	75.0	39	5,700	17.6	1.0	1,569	14.3	815	1.28 × 10^6	1.4
W. S., 55, normal	118 88 × 10^6	82.0	74	6,200	18.5	1.0	1,599	15.2	942	1.5 × 10^6	1.7
A. S., thalassemia major	100 75 × 10^6	36.5	20	2,920	4.32	0.49	618	6.7	196	0.12 × 10^6	0.16
A. L.											
(1)	100 75 × 10^6	35.0	10	2,800	7.1	0.35	585	9.2	258	0.15 × 10^6	0.2
(2)‡	100 75 × 10^6	31.5	13	2,500	2.1			7.9	197		
D. G., thalassemia major	98 73 × 10^6	21.0	21	1,680	4.1	0.24	370	5.1	86	0.032 × 10^6	0.04
Dog	141 105 × 10^6 (by injection)	13.6	6	1,260	218.0	12.6	19,620	9.0	113	2.22 × 10^6	2.1

* Using an average value of 76 ml./kg. for adult males, from data of various authors, quoted by Mollison 1956 [*41*]; 80 ml./kg. for children, an average value from the data of Russell 1949 [*42*]; and 93 ml./kg for the dog, an average from various authors quoted by Wintrobe 1956 [*43*].

† Observed specific activity, has been corrected for background activity and self-absorption, but not for falling hematocrit.

‡ In the second study on A. L., radioactivity was measured in the protoporphyrin of hemoglobin but not in the globin fraction.

in counts/minute/milligram at infinite thinness. By our methods, 1 μc. glycine-2-C^{14} gave 750,000 counts/minute.

Calculations. The expression, counts/minute/milligram, was used for calculation of the total uptake into hemoglobin, and for construction of the red cell survival curves. This expression was converted into terms of counts/minute/millimole of glycine in heme or globin, as follows:

$$(1)\quad \text{counts/minute/millimole of glycine in heme} = \text{counts/minute/milligram protoporphyrin} \times \frac{\text{molecular weight of protoporphyrin methyl ester}}{8}$$

$$(2)\quad \text{counts/minute/millimole of glycine in globin} = \text{counts/minute/milligram globin} \times \frac{\text{molecular weight of globin (65,400)}}{42}$$

This method of calculation was used by Kassenaar, Morell and London [*38*], and by ourselves [*1*] for *in vitro* experiments. The ratio of (1) to (2) is the heme-globin ratio.

RESULTS

The Red Cell Survival Curve. The curves of radioactivity of protoporphyrin in Figure 1 represent the appearance, survival and disappearance of a group of red cells containing C^{14}-labeled hemoglobin. The curves for the normal subjects correspond well with those already published in the literature [*4,12,14,15*]. The normal subject is in a steady state, his red cell mass maintained constant by the even balance between red cell destruction and production. The patients with thalassemia studied, however, were not in a steady state, since their hematocrits fell progressively during the period of investigation, until artificially raised again by transfusion. The fall in hematocrit is probably mainly due to the progressive destruction of aging transfused cells; except during the period immediately following transfusion, the patients' own rates of cell production and destruction were probably approximately constant [*33*]. It seems reasonable to apply an approximate correction for the falling hematocrit

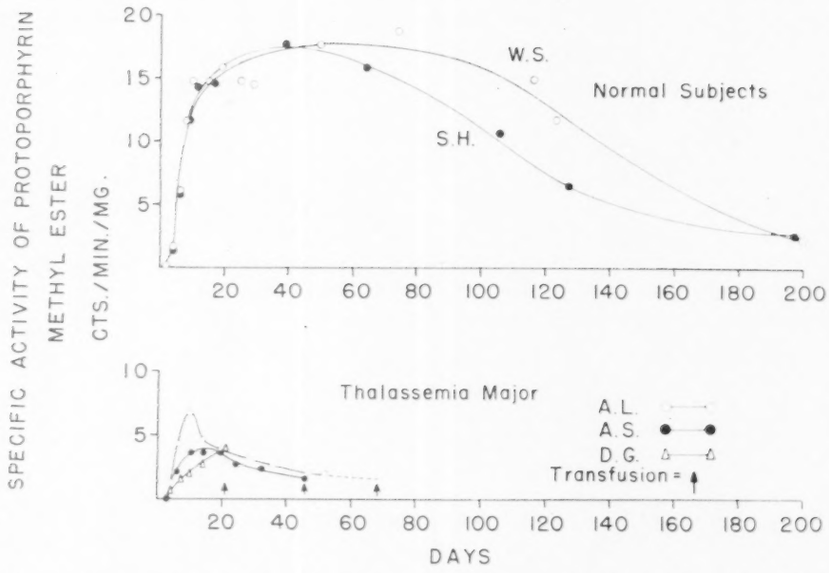


FIG. 1. Curves of specific activity of protoporphyrin of circulating hemoglobin in two normal subjects and in three patients with thalassemia major. A tracer dose of approximately 100 μc. of glycine-2-C^{14} was dissolved in 100 ml. of water and given orally in nine doses over a period of three days.

but he data are presented with and without such a correction. The calculation was made as follows:

$$\text{corrected counts/minute/milligram, day t} = \text{observed counts/minute/milligram, day t} \times \frac{\text{hematocrit on day t}}{\text{hematocrit on day 1}}$$

A similar correction was used for the acute increase of hematocrit *after* transfusion, on the twenty-third day of the second investigation in Case II. Corrected values in Cases I and II (Table III) were used in constructing Figure 1; the corrections are shown in greater detail in Figure 2. Data from Case III were scanty, and this correction was not applied to them. More frequent determinations were not made because the children were so severely anemic and because a minimum of 10 ml. of blood was needed to prepare samples of protoporphyrin and globin.

The form of the thalassemic curves is complex (see "Comments") and although there is evidently marked shortening of cell survival, the mean cell survival time cannot be estimated directly. An index of cell survival is provided by the 50 per cent survival time, which is obtained by drawing a horizontal line across each curve at 50 per cent of the maximum activity and measuring the distance between the points at which this line cuts the ascending and descending limbs of the curve. Using this simple graphic method on the corrected curves in Figure 2, the 50 per cent survival time was thirty-six days in Case I and twenty-eight days in Case II. The radioactivity of circulating hemoglobin in the second investigation in Case II was extremely low, probably because of the severity of the patient's illness, and because of the depressant and diluting effect of a recent transfusion; the radioactivity curve was not corrected and is used in Figure 3 only to provide a comparison for the radioactivity of the stercobilin. Data from the third patient with thalassemia (Case III) provide no information about his red cell survival, since the plateau of activity had not been reached before transfusion was necessary, and after transfusion the activity was too low for accurate counting.

The normal subjects' 50 per cent survival times were 118 and 128 days; for normal subjects the 50 per cent survival time is a more valid approximation to the mean cell survival time.

The Comparative Labeling of Heme and Globin. The specific activities of heme and globin of each subject, followed for 200 days in the normal subject and until transfusion was necessary in the patients with thalassemia, and of the dog, are shown in Table IV. The values in this table are not corrected for falling hematocrit, since such

TABLE III
SPECIFIC ACTIVITY OF PROTOPORPHYRIN OF CIRCULATING HEMOGLOBIN (CORRECTED FOR FALLING HEMATOCRIT)

Subject	Day of Investigation	Observed (ct./min./mg.)*	Corrected (ct./min./mg.)
A. S.	3	0.06	0.06
	6	2.2	2.12
	11	3.73	3.44
	15	3.86	3.44
	20	4.32	3.71
	25	3.43	2.84
	33	3.1	2.34
	46	2.65	1.77
A. L.	4	1.4	1.37
	7	4.4	4.22
	10	7.1	6.7
	14	5.0	4.63
	20	4.25	3.84
	29	3.5	3.1
	36	3.2	2.63
	47	2.86	2.17
	52	2.9	2.14

* Observed specific activity, corrected only for background activity and self-absorption.

a correction is unnecessary in comparing the activity of heme and globin. Radioactivity is also expressed in counts/minute/millimole of glycine in heme or globin; the heme:globin ratio was calculated as already described.

The mean value of the heme:globin ratio was 0.784 in each of the two normal subjects. The values after about day 100 were definitely lower than the mean; while the number of determinations was small, the fall seemed to be progressive, indicating a relative increase in globin radioactivity as compared with that of heme.

The ratios were lower throughout the period of investigation in the patients with thalassemia: those for A. S. (Case I, 0.494) and A. L. (Case II, 0.614) being significantly lower than the normal when compared by the t test ($p < 0.001$ and $p < 0.02$, respectively). A fall in the ratios towards the end of the period of investigation, as noted in the normal subjects, was observed for A. L. but not A. S. The third patient (Case III) had a mean ratio of 0.740, not significantly lower than the normal; this ratio however is based on only four values, before the peak of activity was reached. It is emphasized that the thalassemic samples were of relatively low activity (Tables III and IV), giving low counting rates so that their relative accuracy is less. The standard deviation of counting at rates twice background with our equipment is 0.4 counts per minute and the standard error is 0.12.

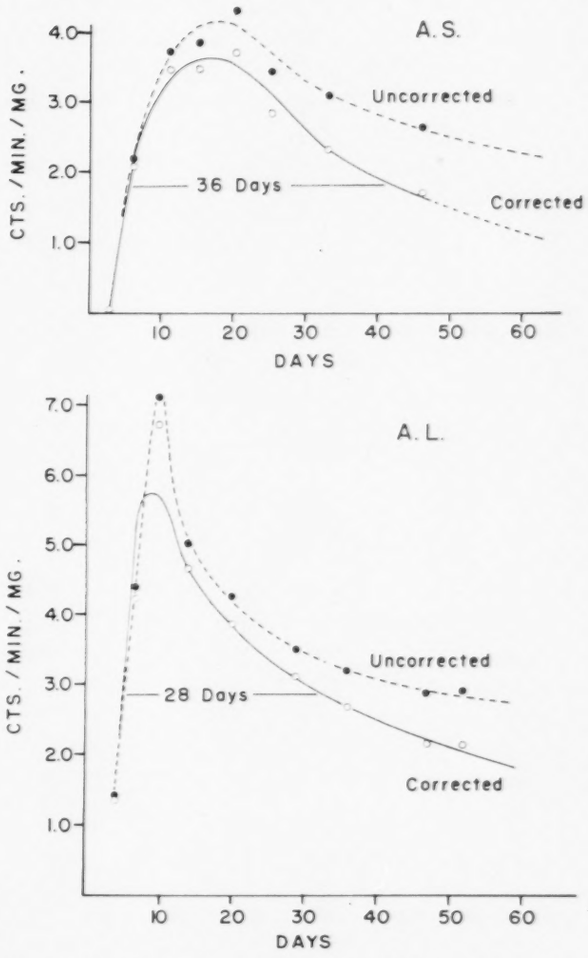


FIG. 2. Specific activity of protoporphyrin of circulating hemoglobin, corrected for falling hematocrit, in two patients with thalassemia major. See Table II for corrected figures, and text for discussion of the calculation.

The ratio in the dog was somewhat higher, the mean value being 0.842.

Extent of Utilization of Glycine for Hemoglobin Synthesis. Utilization has been calculated from the data recorded in Table II. The basic data used were the subjects' weights, the dose of glycine administered, and the maximum radioactivity observed in the protoporphyrin and globin of circulating hemoglobin. The total circulating hemoglobin was derived from the observed hemoglobin level on the appropriate day of study and an assumed blood volume based on body weight (see note below Table II).

TABLE IV

RADIOACTIVITY OF PROTOPORPHYRIN AND GLOBIN OF CIRCULATING HEMOGLOBIN, AFTER ORAL ADMINISTRATION OF 100 μC. GLYCINE-2-C^{14} (HEME:GLOBIN RATIO)*

Subject, Age (yr.) and Weight (kg.)	Day	Observed Specific Activity† (ct./min./mg.)		Activity Expressed in ct./min./mM of Glycine in		Heme:Globin Ratio
		Protoporphyrin Methyl Ester	Globin	Heme (Protoporphyrin)	Globin	
S. H. (normal adult), 55, 75	4	1.3	0.07	100	114	0.87
	6	5.8	0.3	427	460	0.93
	9	11.7	0.7	860	1,075	0.80
	12	14.2	0.87	1,044	1,350	0.78
	17	14.5	0.80	1,066	1,245	0.86
	39	17.6	1.0	1,295	1,560	0.83
	64	15.6	0.84	1,148	1,310	0.88
	106	10.7	0.74	790	1,145	0.69
	127	9.0	0.63	664	985	0.68
	197	2.5	0.17	139	265	0.52
						(Mean 0.784 ± 0.336‡)
W. S. (normal adult), 55, 82	2	0.2	0	15	0	
	4	1.6	0.1	114	156	0.73
	6	6.0	0.28	440	437	1.0
	8	11.6	0.58	855	905	0.94
	10	14.8	1.0	1,090	1,560	0.7
	15	14.75	0.8	1,085	1,245	0.87
	19	15.8	0.82	1,162	1,270	0.92
	25	14.7	0.91	1,082	1,420	0.76
	29	14.4	0.95	1,060	1,470	0.72
	50	17.5	1.11	1,290	1,730	0.75
	74	18.5	1.1	1,360	1,690	0.8
	116	14.8	1.0	1,090	1,560	0.7
	123	11.6	1.05	856	1,640	0.52
	200	2.4				
						(Mean 0.784 ± 0.038‡)
A. S. (thalassemia major), 14, 36.5	3	0.06	0	5		
	6	2.2	0.28	162	443	0.37
	11	3.73	0.31	275	484	0.57
	15	3.86	0.38	284	586	0.49
	20	4.32	0.49	318	768	0.41
	25	3.43	0.29	255	452	0.56
	33	3.1	0.28	228	437	0.52
	40		0.26		406	
	46	2.65	0.23	195	359	0.54
	46	Transfusion				(Mean 0.494 ± 0.029‡)
A. L. (thalassemia major), 10, 35	4	1.4	0.08	102	125	0.82
	7	4.4	0.4	324	616	0.53
	10	7.1	0.36	525	557	0.94
	14	5.0	0.38	371	600	0.62
	20	4.25	0.34	313	534	0.59
	29	3.5	0.31	258	484	0.53
	36	3.2	0.31	233	484	0.48
	47	2.86	0.26	211	407	0.52
	52	2.9	0.29	214	432	0.5
	67	Transfusion				(Mean 0.614 ± 0.053‡)

TABLE IV (*Continued*)

RADIOACTIVITY OF PROTOPORPHYRIN AND GLOBIN OF CIRCULATING HEMOGLOBIN, AFTER ORAL ADMINISTRATION OF 100 μC. GLYCINE-2-C^{14} (HEME:GLOBIN RATIO)*

Subject, Age (yr.) and Weight (kg.)	Day	Observed Specific Activity† (ct./min./mg.)		Activity Expressed in ct./min./mM of Glycine in		Heme:Globin Ratio
		Protoporphyrin Methyl Ester	Globin	Heme (Protoporphyrin)	Globin	
D. G. (thalassemia major), 8, 21	4	0.74	0	55		
	7	1.45	0.086	107	135	0.79
	10	1.98	0.20	146	300	0.49
	14	2.61	0.17	192	260	0.74
	21	4.10	0.21	302	320	0.94
	21	Transfusion				(Mean 0.740 ± 0.094‡)
Male dog, 13.5, (140 μc. by injection)	4	170	8.8	12,500	13,700	0.91
	6	218	12.6	16,100	19,650	0.82
	8	200	11.8	14,700	18,600	0.78
	12	164	9.3	12,200	14,500	0.84
	22	126	6.9	9,300	10,750	0.86
						(Mean 0.842 ± 0.01‡)

* Heme:globin ratio is calculated as described in the text. Data on A. L. (Case II) refer to the first investigation on this patient.

† Observed specific activity, corrected only for background activity and self-absorption, not for falling hematocrit.

‡ Standard error of the mean.

The specific activity of hemoglobin as a whole was obtained by adding the individual activities of protoporphyrin and globin in correct proportion for a unit weight of hemoglobin, assuming that heme comprises 3.6 per cent and globin 96.4 per cent, by weight, of hemoglobin.

It will be observed from Table II and Figure 1 that the proportion of the total dose utilized in the synthesis of circulating hemoglobin is considerably less in patients with thalassemia (0.16 and 0.2 per cent) than in normal subjects (1.4 and 1.7 per cent). It was still lower in the second investigation in Case II, as already mentioned, but the calculated per cent utilization is not presented here because of the inaccuracy of the low counting rate and the lack of globin data in this investigation.

The Excretion and Radioactivity of Fecal Urobilinogen. These observations were made during the second investigation in Case II. Daily urobilinogen excretion, and the radioactivity of stercobilin and of the protoporphyrin of circulating hemoglobin are shown in Figure 3, which illustrates the results for thirty-four days. The patient received a blood transfusion five days before the oral dose of glycine-2-C^{14}, and another was necessary on the twenty-third day of the investigation.

Excretion of urobilinogen varied from day to day, and averaged about 200 mg. per day over the whole period. Radioactivity appeared in stercobilin within twenty-four hours of the initial administration of glycine-2-C^{14} and increased rapidly, to reach a peak of activity on the third day. At this time its activity was over 100 times greater than the maximum activity observed in the protoporphyrin. Activity thereafter declined by an exponential curve, probably consisting of two components. Protoporphyrin activity reached a maximum on the eleventh day in this study, but as already mentioned the incorporation was extremely low, and only about one-fifth of that found in the first investigation on this patient.

COMMENTS

An interesting and unexpected finding in this study was the much lower radioactivity observed in hemoglobin in the children with thalassemia major than in the normal adults (Fig. 1), al-

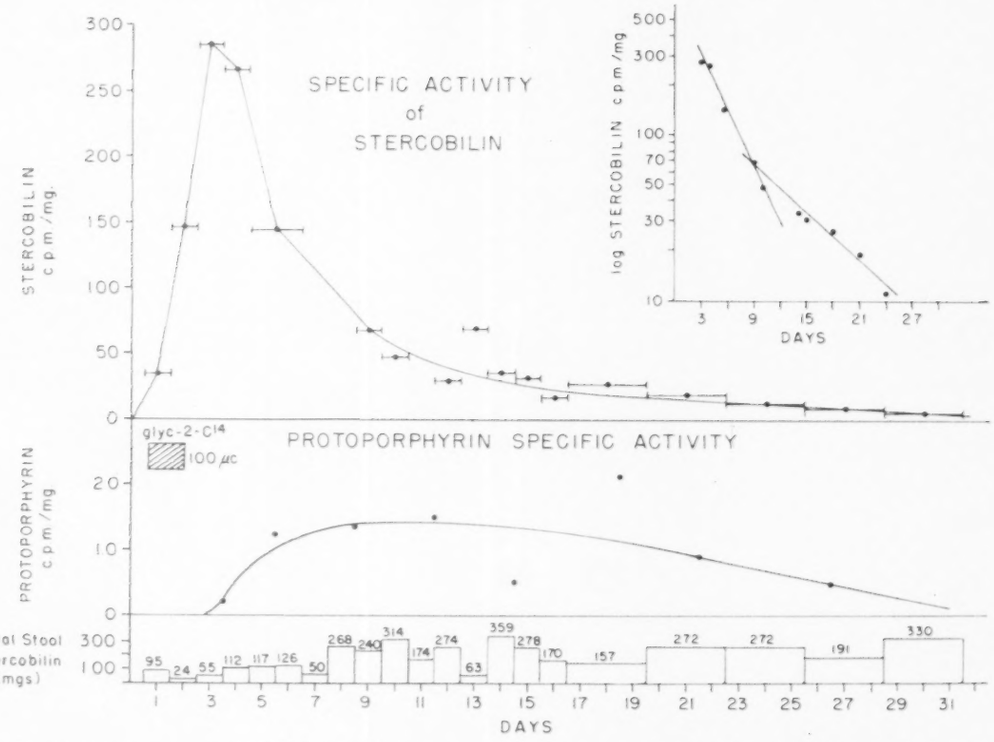


FIG. 3. Radioactivity in stercobilin and in the protoporphyrin of circulating hemoglobin after oral administration of glycine-2-C^{14} to patient with thalassemia major. Radioactivity appeared in stercobilin within twenty-four hours, and increased rapidly to reach a peak activity more than 100 times greater than the maximum activity observed in the protoporphyrin of circulating hemoglobin. The insert demonstrates that the exponential curve expressing the decline in stercobilin activity may consist of two components.

though each received the same tracer dose of 100 μc. The result is in contrast to what has been observed in other diseases in which there is an increased erythropoietic demand, such as polycythemia [*12*] and sickle cell anemia [*12,44*]. (Fig. 4.) This apparently lower utilization is difficult to interpret, since quantitative investigations by Sturgeon and Finch [*26*], and the recent more extensive studies of Erlandson and her colleagues [*45*], show that the marrow's erythropoietic output is normal or increased in thalassemia major, although the increase is not sufficient to compensate for the degree of shortening of red cell life. The turnover of glycine for hemoglobin synthesis must therefore be rapid, and it would have been expected that the labeling of heme would be at least as great as in the normal subject. One possible explanation for the low radioactivity is provided, if it is postulated (following Sturgeon and Finch) that much more hemoglobin (or a precursor) is manufactured in the bone marrow than finally enters the peripheral blood; the C^{14} label would thus be spread thin over a large amount of hemoglobin (or precursor) and so would appear much diluted in the heme of circulating hemoglobin.

The time of appearance of radioactivity in heme was surprisingly delayed in these patients; the maximum was not reached until the tenth day or later, whereas in sickle cell anemia, using N^{15}-glycine, maximum isotope content has been demonstrated on the seventh day or earlier [*12,44*]. Larrizza and his colleagues [*46*] have observed the same phenomenon in their investigations using Fe^{59}, in which maximum radioactivity in circulating hemoglobin was reached later in thalassemia than in iron deficiency anemia, hereditary spherocytosis and pernicious anemia. These observations suggest that there is a relative delay in the maturation and release of erythrocytes—a view supported by morphologic observations, particularly those of Astaldi et al. [*47,48*].

Following the delayed appearance of radioactivity in heme, no plateau of activity was observed. (Fig. 2.) The descending curves are

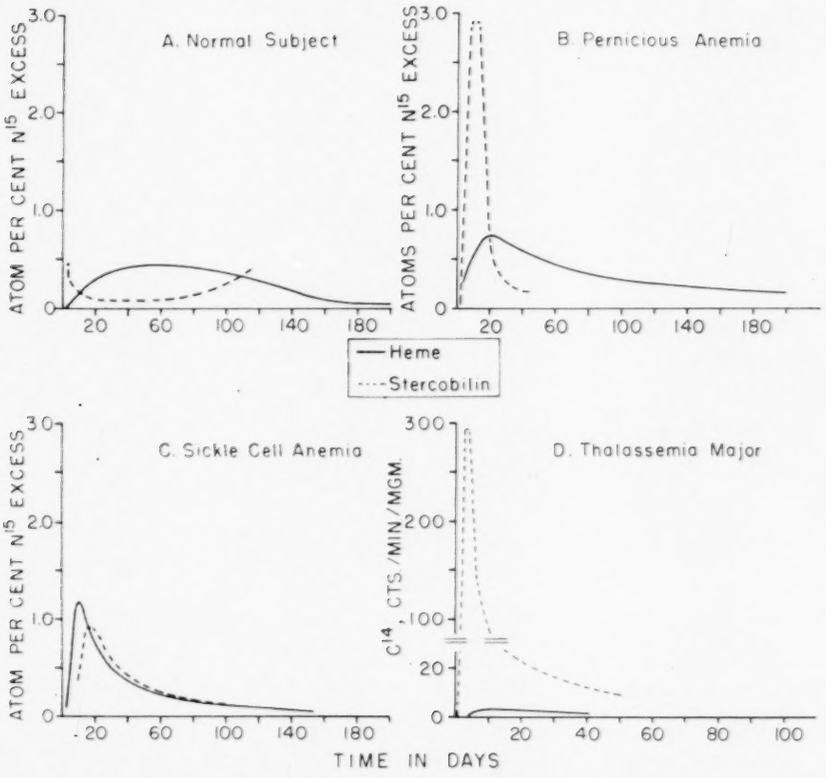


FIG. 4. Diagrammatic comparison of the dynamic relation between the labeling of heme and stercobilin after the administration of a tracer dose of tagged glycine. A, normal subject, after London et al. [*19*]. B, subject with pernicious anemia, after London and West [*23*]. C, subject with sickle cell anemia, after London et al. [*12*] and James and Abbott [*44*]. D, subject with thalassemia major, from Figure 3. Note that diagram D is from a study in which C^{14} was the tag, rather than N^{15} as in the other instances. In the conversion of heme to stercobilin there is no loss of labeled N atoms derived from glycine, but one-eighth of the labeled C atoms derived from glycine are lost. If the specific activity in curve D were corrected for this loss, the difference between the labeling of heme and of stercobilin would be further emphasized.

not precisely exponential in form, and when plotted on a semi-logarithmic scale give lines convex to the time axis. Such curves are compatible with a process of random destruction with considerable heterogeneity in the red cell population. Considering the remarkable variation in the morphology of the red cells in thalassemia major, heterogeneity in survival is not unexpected. The result for the functioning of the individual patient is a moderately severe hemolytic process (50 per cent survival times of thirty-six and twenty-eight days, respectively) with increased demand upon the bone marrow. Shortened red cell survival has previously been demonstrated by the method of differential agglutination [*49–51*] and by Cr^{51} tagging [*26,45,52*]. In the present investigation the degree of shortening of survival corresponded roughly with the clinical severity of the disease; A. S. (Case I, thirty-six days) was less severely affected than A. L. (Case II, twenty-eight days).

Surprisingly high radioactivity was found in the stercobilin within a few days after administration of glycine-2-C^{14}, reaching a maximum on the third day, when the activity was over 100 times greater than that of the circulating heme. Both the time of appearance of the early labeling, and the actual activity indicate that much of this stercobilin was not derived from the breakdown of *circulating* hemoglobin. The descending part of the stercobilin curve (Fig. 3) may be resolved into two components when plotted on a semi-logarithmic scale against time (insert of Fig. 3), suggesting that it is derived from at least two sources. The second of them, corresponding to the shallower part of the curve,

may be due to destruction of circulating hemoglobin, since on the semi-logarithmic scale it gives a straight line approximately parallel to that of the declining activity of heme. The peak plus the first and more rapid component of the descending curve might be derived from one or more of the possible sources previously mentioned, namely destruction of hemoglobin in the bone marrow, or possibly an anabolic process in which stercobilin might be formed from a pyrrol compound other than heme.

The high early radioactivity of stercobilin in thalassemia major is a great exaggeration of what has been observed in pernicious anemia [*23*]. The pattern is different from that found in sickle cell anemia [*44*] in which the high stercobilin activity seems to follow the peak activity in the heme. (Fig. 4.)

The interpretation of the heme:globin ratios is difficult, since the normal relationship between heme and globin biosynthesis is not yet clear. *In vivo* in the rat and rabbit Neuberger and his colleagues [*17,18*] found a relatively constant heme:globin ratio except in the first twenty-four hours after administration of isotopes. The problem has recently been discussed by Rimington [*53*]. Calculated by the method used in the present investigation, the theoretic ratio, if heme and globin each incorporated their molecular fair share of glycine from the same pool, would be unity. There is evidence that globin may receive an extra contribution by transformation of amino acids, in particular by conversion of glycine to serine [*54*], which would tend to lower the ratio.

In the present investigation the mean ratio found in both the normal subjects was 0.784. Further, the ratios were consistently lower than the mean value after the 100th day. This corresponds to the period during and after the destruction of the original population of labeled erythrocytes, when persistent isotope-labeling of hemoglobin forming a "tail" to the curve, has been noted by many previous investigators. This tail has been ascribed to re-utilization of C^{14} (or N^{15} in some investigations), or to the persistence of some of the original dose of labeled glycine in the glycine pool for an unexpectedly long time [*55*]; a third possibility, that some erythrocytes survive much longer than 120 days seems the least likely. The fall in heme:globin ratio during the tail period indicates relatively more C^{14} in globin than in heme. Since it is considered that the heme moiety of hemoglobin is entirely excreted as bile pigment when the red cell is destroyed, if C^{14} is re-utilized it must be derived from globin only. The fall in the heme:globin ratio suggests that, if there is re-utilization, a larger share of the C^{14} goes to globin, perhaps because sub-units larger than the glycine molecule and derived from the breakdown of globin are more easily re-utilized for the synthesis of globin.

A significantly lower than normal mean heme:globin ratio was observed in two of the three patients with thalassemia who were studied. In previous *in vitro* investigations of hemoglobin synthesis in thalassemia [*1*], the range observed was wide but no consistent difference was noted between the heme:globin ratio of newly synthesized hemoglobin of blood from patients with thalassemia and that of blood from patients with other forms of hemolytic anemia, the mean being around 1. It was therefore concluded at that time that there was unlikely to be a specific abnormality in globin synthesis. The finding of a significantly lower ratio *in vivo* is difficult to interpret, and may remain so until the normal interrelationship is understood. It is possible that because of the rapid rate of red cell destruction there is considerably more reutilization of C^{14} with resultant lowering of the ratio by preferential re-utilization for globin synthesis, as already postulated.

In the present investigation no attempt was made to distinguish between the metabolism of normal adult and alkali-resistant (fetal) hemoglobin, although two of the patients had considerable amounts of the latter. (Table 1.) It would be of great interest to separate the hemoglobins in such an investigation, since there is evidence that their metabolism may sometimes be "heterogeneous" [*56*]. In a recent study, however, no difference was detected in the Fe^{59} specific activity of different hemoglobin fractions from a series of subjects, including one with thalassemia major [*57*].

Concluding Remarks. The investigations reported here thus show that the C^{14} tagging of the end product, hemoglobin, is greatly reduced and delayed in thalassemia, whereas the tagging of stercobilin—a catabolic product, or perhaps a by-product—is greatly increased and appears early. These observations are compatible with the general concept that: (1) much of the erythroblastic activity in this disorder is ineffective in delivering an adequate supply of erythrocytes to the peripheral blood; and (2) a large portion

of the heme (or precursor or other pyrrol compound) synthesized is diverted into an abortive pathway, resulting in the production of excess stercobilin. This latter process must be a rapid one, since maximum stercobilin radioactivity occurred on the third day.

In vitro experiments [7] provided evidence of the quantitative impairment of hemoglobin synthesis by the *individual* immature cell, probably because of a defect in heme synthesis. *In vivo* studies measure the activity of all these apparently defective units together. The erythropoietic system in thalassemia major may be visualized as being composed of a greatly increased number of erythroblasts; they are producing, too slowly and in too few numbers, incompletely hemoglobinized erythrocytes with impaired capacity for survival. They are probably the source of the excess quantities of bile pigment, perhaps derived from the early breakdown of erythrocytes or failure to convert all the porphyrin formed to hemoglobin. It may be possible to trace all these abnormalities to a basic enzyme defect in the erythroblasts—the biochemical lesion of thalassemia—determined by the thalassemia gene, acting in double dose. Further investigations are in progress to try to pinpoint the lesion.

SUMMARY

1. C^{14}-labeling of heme and globin was measured in three patients with thalassemia major and in two normal subjects after the oral administration of 100 μc. of glycine-2-C^{14}. The labeling of stercobilin also was measured in one patient with thalassemia.

2. In the patients with thalassemia, the apparent utilization of glycine for hemoglobin synthesis was decreased, and the appearance of radioactivity in circulating hemoglobin was relatively delayed. In contrast, radioactivity appeared extremely rapidly in the fecal stercobilin, reaching a maximum on the third day, when it was over 100 times greater than the maximum radioactivity found in heme.

3. The 50 per cent red cell survival was estimated in two patients with thalassemia as thirty-six and twenty-eight days, and in two normal subjects as 118 and 128 days.

4. The relative labeling of heme and globin was expressed as the heme:globin ratio, which had a mean value of 0.784 in both normal subjects, and fell after the 100th day; it is suggested that the fall may be due to preferential re-utilization of C^{14} for globin synthesis. The ratio was significantly lower than normal in two of the three patients with thalassemia.

5. These results are compatible with the concept that there is a defect in hemoglobin synthesis as well as an abnormality of erythrocyte production in thalassemia major. The enhanced early labeling of stercobilin implies an abnormal degree of premature destruction of red cells or hemoglobin within the bone marrow, or an anabolic pathway for the production of stercobilin. The basic abnormality would seem to be in the erythroblast, a genetic biochemical lesion, it is suggested, not yet identified.

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APPENDIX

CASE 1. The diagnosis of thalassemia major was made in A. S., a fourteen year old girl, during her infancy. Splenomegaly and trabeculation of the bones on roentgenologic examination were first noted at the age of six months. She required repeated transfusions. At the age of eleven, splenectomy was performed at another hospital because her transfusion requirements had increased considerably. The spleen, which weighed 750 gm., had histologic changes of reticular hyperplasia, areas of hemopoietic tissue and increased hemosiderin deposition. A biopsy specimen of the liver also showed abundant hemosiderin, with moderate destruction of parenchymal architecture and increase in portal fibrous tissue. After splenectomy, transfusions were required less frequently, and at the time of study were being given at intervals of two to three months.

The patient was a slender, olive-gray skinned girl, with slight conjunctival icterus. Development of the breast was normal for fourteen years, and the menarche occurred while the study was in progress. Representative hematologic data before transfusion

are shown in Table I. There was marked anemia with hypochromia and anisocytosis, with many reticulocytes and normoblasts in the peripheral blood.

Both parents, of Italian ancestry, have the characteristic microcytosis and hypochromia, with normal serum iron, of the thalassemia trait. The subject's twin brother died of pneumonia at six weeks of age. It is not known whether he, too, was anemic. Another younger sibling, said to be a "blue baby," died seven days after birth. The younger siblings are healthy; neither has splenomegaly nor anemia; their blood was not examined in detail. A first cousin with thalassemia major died at thirteen years of age.

CASE II. A. L., ten year old girl, was first referred to another hospital at the age of seventeen months with bronchitis. At that time, she was found to have hepatosplenomegaly, with microcytic hypochromic anemia and immature red cells in the peripheral blood. Thalassemia major was diagnosed and treated with blood transfusion. Splenectomy was performed. During the succeeding years she received repeated transfusions; her skin became much darker; and marked hepatic and cardiac enlargement occurred. Characteristic roentgenological changes in the bones were noted at six years of age. Cardiac failure developed at eight years.

At the time of the first investigation in July 1957, she was a small sallow girl whose protruberant abdomen contrasted with her thin limbs. She had characteristic facial changes with high cheekbones and prominent upper incisor teeth. There was marked slate brown pigmentation of the skin. The edge of a large firm liver was palpable 12 cm. below the right costal margin. Hematologic data are recorded in Table I.

By September 1958 her condition had deteriorated, and she was in hospital with severe cardiac failure throughout the period of the second isotope investigation (October 1958). The seriousness of the illness restricted the extent of investigation and may have affected hemoglobin metabolism. She remained in chronic congestive heart failure and died on March 6, 1959.

Both parents, of Italian ancestry, showed the changes of the thalassemia trait. (Table I.) Two younger siblings were apparently healthy, and their blood was reported to be normal.

CASE III. D. G., an eight year old boy, had severe thalassemia major. The diagnosis was made at four months of age when he was found to have microcytic, hypochromic anemia (hemoglobin 4.5 gm./100 ml.) with enlargement of liver and spleen. He required repeated blood transfusions.

At the time of study he was a small, thin, pale, dark-skinned boy with a protruberant abdomen, high forehead and cheekbones, and prominent upper incisor teeth. The liver was enlarged to 7 cm. and the spleen to 11 cm. below the costal margin. Hematologic data are included in Table I. His transfusion requirements had increased so that at the time of study he was receiving transfusions at intervals of two to three weeks to maintain the hemoglobin above 5 gm./100 ml. Cr^{51} red cell survival study showed a Cr^{51} half-time of seven days. Marrow aspirated at this time was hypocellular. Reticulocyte counts were repeatedly less than 0.1 per cent; no normoblasts were seen in peripheral blood films; the platelet count was only 22,000/cu. mm. These findings together with the presence of a large spleen suggested that there was secondary dysplenism accounting for the increased rate of destruction of his own and transfused blood, in addition to relative marrow failure. At the time of the glycine-2-C^{14} study (July 1957) he appeared to be making little blood of his own, and rapidly destroying that which he received via transfusions.

Splenectomy was therefore performed shortly after the investigation, and was followed by the appearance of immature cells in the peripheral blood, and by marked diminution in transfusion requirements. The remission continued for some six months, until March 1958, when he was readmitted to the hospital with pyrexia and cardiac failure. Repeated blood transfusions were again required; despite these, and treatment with digitalis and antibiotics, the patient remained extremely ill, and finally died in September 1958. At autopsy there was gross siderosis of skin and viscera particularly of the gastrointestinal mucosa, ascites and pericardial effusion, focal hepatic fibrosis and terminal jaundice.

Both parents, of Italian ancestry, showed the characteristic changes of the thalassemia trait. (Table I.) There were no siblings, and no history of anemia in other relatives.

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Familial Hypogammaglobulinemia, Splenomegaly and Leukopenia*

With a Review of the Etiological Factors of the Hypogammaglobulinemias

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THE description of congenital agammaglobulinemia by Bruton in 1952 [*1*] was followed by clinical recognition of "acquired" idiopathic hypogammaglobulinemia and transient physiological hypogammaglobulinemia of infancy, and by renewed interest in hypogammaglobulinemia associated with certain other diseases, such as the nephrotic syndrome and multiple myeloma [*2*]. The etiologies of these forms of hypogammaglobulinemia have yet to be fully elucidated. It is the purpose of this paper to describe a new clinical entity in which hypogammaglobulinemia associated with leukopenia and splenomegaly appears as a familial disturbance perhaps transmitted by an autosomal dominant gene. In addition, the known etiological factors of the hypogammaglobulinemias will be reviewed and commented upon.

CASE REPORTS

CASE 1. The proband, a forty-two year old white man, was seen at the Research Clinic of the Hektoen Institute for Medical Research of the Cook County Hospital in May 1957. His chief complaint was chronic, multiple ulcers of the right leg and recurrent fever of three years' duration. He had had "bronchial pneumonia" when two and a half years old and "flu pneumonia" at five years. When he was five, a tonsillectomy and adenoidectomy were performed. He had chicken pox at six years and measles at seven. At ten, a third episode of pneumonia occurred. Throughout childhood and adolescence he had frequent infections of the upper respiratory tract, "styes," "pink eyes" and earaches. "Boils" and abscesses occurred frequently after trauma and along the "belt line;" some required incision and drainage. In late adolescence chronic sinusitis developed. He had a fourth episode of pneumonia at twenty-one years of age. Also at twenty-one he injured his right knee while playing college football. Subsequently, mild aches and slight swelling of this knee frequently recurred. During limited service with the armed forces, when twenty-seven to twenty-nine years old, total dental extraction for caries and pyorrhea was followed by mandibular osteomyelitis; he was also treated for a thumb infection. At this time a roentgenogram of the chest and a hemogram were within normal limits.

Despite these numerous illnesses he developed normally into a large athletic man, who fathered seven children. From the age of thirty-eight, however, until his death four years later he was in chronic ill health. The first episode of an illness characterized by recurrent chills and fever, with temperatures as high as 104°F., fatigue, and cough productive of green sputum occurred when the patient was thirty-eight years old. A roentgenogram of the chest showed right costophrenic angle obliteration and a small area of platelike atelectasis at the base of the left lung. Previous roentgenograms taken in 1950 and 1951 had not demonstrated any abnormalities. The illness lasted seven months and the patient improved after a one-month course of penicillin. During this illness (June 1953) painless, pea-sized subcutaneous nodules appeared on the posterior surface of his right leg. These subsequently ulcerated and drained thick yellow material.

In March 1954 he entered another clinic complaining of ulcers of the leg, cough, weakness and episodes of chills and fever. Physical examination showed a well nourished man with numerous subcutaneous

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TABLE 1

FAMILIAL HEMATOLOGICAL DATA

Case No.	Date	Red Blood Cells (per cu. mm.)	Hemoglobin (gm./100 ml.)	Platelets (per cu. mm.)	White Blood Cells						
					Total (per cu. mm.)	Polymorphonuclears (%)	Band Forms (%)	Eosinophils (%)	Basophils (%)	Lymphocytes (%)	Monocytes (%)
Patients with Hypogammaglobulinemia											
1	3/54	4,180,000	11.7	...	1,200	...	...	...	...	...	...
	4/57	...	13.0	Normal	4,000	69	...	2	...	28	1
	12/57	3,820,000	10.0	...	5,000	52	...	...	...	47	1
	2/58	...	...	Normal	3,750	58	14	12	...	8	8
	5/58	4,520,000	13.4	...	2,800	64	12	6	...	14	4
	7/58	...	13.4	...	2,400	...	...	...	...	...	...
2	3/54	4,540,000	12.2	...	1,800	65	...	13	1	20	1
	4/57	...	12.8	Normal	2,200	42	27	1	1	16	13
	4/59	...	14.0	630,000	3,300	73	5	1	1	11	9
3	9/58	4,200,000	12.5	Normal	3,000	54	22	3	1	16	4
	4/59	...	12.0	511,200	2,950	69	8	...	...	16	7
4	9/58	4,000,000	11.7	Normal	3,900	49	25	4	1	14	7
	4/59	...	12.6	720,000	1,650	62	15	1	3	19	10
Normal Subjects											
5	6/59	3,900,000	10.0	Increased	5,000	61	8	1	...	20	10
6	6/59	5,100,000	14.4	Normal	6,900	42	8	...	...	40	10
7	6/59	5,000,000	13.8	Normal	6,500	55	5	...	...	30	10
8	6/59	4,750,000	13.4	Normal	10,150	53	5	4	2	31	5
9	11/58	5,110,000	15.1	Normal	8,550	72	3	...	...	20	5
10	11/58	5,490,000	14.3	Normal	9,050	59	2	...	...	36	3
11	11/58	6,520,000	19.6	Normal	7,500	66	1	...	...	26	7

nodules and one draining sinus of the right leg. Urinalysis was negative. The hemoglobin was 11.7 gm. per cent, erythrocytes numbered 4,180,000 per cu. mm. and the leukocytes 1,200 per cu. mm. (Table 1.) The blood smear showed "nothing that was considered diagnostic." Roentgenograms demonstrated thickening of the sinus membranes, osteoporosis about the right knee joint, and an irregular density with linear strands in the upper lobe of the left lung. Bronchoscopy revealed no abnormalities. Cultures for bacteria, fungi and tubercle bacilli from the bronchial secretions and the ulcers of the leg were all negative, as were guinea pig inoculations. The histoplasmin skin test was positive.

In December 1954 the patient entered a local hospital for one week. The diagnosis on discharge was acute pharyngitis; paronychia of the right index finger; and simple leukopenia with fever, chills and headache. Two weeks later he was again hospitalized because of sudden hemoptysis. Roentgenograms of the chest revealed an infiltration of the upper lobe of the right lung and a large cavity. A fluid level developed, but the cavity gradually healed during one month of combined penicillin-streptomycin therapy. Smears and cultures of the sputum were consistently negative for tubercle bacilli.

For the next two years the patient experienced recurrent chills and fevers, chronic fatigue and occasional episodes of pharyngitis. The ulcers of the leg reappeared with each febrile episode and healed spontaneously between them.

In April 1957 he was referred to one of us (D. S. K.) for investigation with a presumptive diagnosis of chronic active histoplasmosis. Chills, fever and fatigue had persisted for six weeks. His cough was mild and productive of one cup of thick green sputum daily. He also complained of slight dyspnea on exertion.

Upon hospitalization, physical examination revealed a well developed, well nourished man who did not look very ill. The blood pressure was 110/70 mm. Hg, the pulse 56/minute, and the temperature 99.8°F. Significant physical findings included a blunt-edged spleen tip, extending 4 cm. below the left costal margin, a barely palpable liver edge, swelling of the right knee, and multiple, superficial, punched-out ulcers, 1 to 2 cm. in diameter, extending from the knee to the ankle. The skin between the ulcers was bluish and scaling. There were no varicosities or edema. (Fig. 1.)

Urinalysis revealed a pH of 4.5, a specific gravity of 1.021, and negative tests results for protein, sugar and acetone. Microscopy disclosed an occasional erythrocyte and white blood cell. After twelve hours of water deprivation the urine specific gravity was 1.024. Phenolsulfonephthalein excretion was 48 per cent in one hour, 70 per cent in two hours. The serological test for syphilis was negative. The erythrocyte

TABLE II

FAMILIAL BIOCHEMICAL DATA; STUDIES ON SERUM

Case No.	Date	Cholesterol (mg./100 ml.)	Alkaline Phosphatase (Bodansky units)	Icterus Index (units)	Cephalin Flocculation	Thymol Turbidity (units)	Zinc Sulfate Turbidity (units)	Uric Acid (mg./100 ml.)	Mucoprotein (mg./100 ml.)	Total Lipid (mg./100 ml.)	Phospholipid (mg./100 ml.)
Patients with Hypogammaglobulinemia											
1	4/57	178	3.8	9	0	1.0	1.0	10.5	207	660	7.9
	7/58	100	6.2	8	0	0.1	1.2	8.8	260	514	7.8
2	10/57	108	11.4	...	0	1.2	2.3	6.8	275	...	...
	1/59	...	...	8	0	1.0	0.8	...	112	560	7.1
3	1/59	138	5.6	8	0	1.4	1.2	9.0	120	620	7.9
4	1/59	150	10.1	10	0	3.0	1.6	8.0	133	640	8.5
Normal Subjects											
5	9/58	150	2.2	7	2+	3.2	12.2	4.5	108	384	6.0
6	6/59	147	9.6	9	2+	2.6	13.6	5.8	120	435	8.6
7	6/59	127	11.3	8	1+	1.8	11.8	4.9	100	435	8.2
8	6/59	147	10.6	8	2+	2.0	9.2	4.9	90	384	7.1
9	11/58	188	2.4	8	0	1.8	13.8	8.0	116	600	9.9
10	11/58	194	5.9	7	0	1.8	12.9	10.2	125	523	9.3
11	11/58	228	2.3	8	0	2.0	12.2	8.4	142	820	12.2

sedimentation rate was 21 mm./hour. An L.E. cell preparation was negative. Hematologic and biochemical determinations are shown in Tables I and II. The electrocardiogram was normal. Roentgenograms of the chest revealed a fibrotic strand in the upper lobe of the right lung. The remainder of the lung field was clear. A roentgenogram of the abdomen confirmed the presence of a markedly enlarged spleen with no apparent calcifications. The vertebrae were normal. Views of the right knee showed a small tibial exostosis and bony demineralization. Results of skin tests with purified protein derivative No. 1 and No. 2, coccidioidin and blastomycin were all negative. The reaction to the histoplasmin skin test was positive. Cultures of the stool, urine and blood, and fungus cultures of sputums made especially for histoplasma were all negative. Agglutination tests for typhoid, salmonella, brucellosis, heterophil antibody and proteus OX-19 also were negative. A scalene lymph node biopsy revealed an intact capsule, clearly defined peripheral sinuses, a stroma of lymphoid follicles, and a number of fat cells. No plasma cells or granulomas were observed. The serum gamma globulin level (Table III) confirmed the presumptive diagnosis of hypogammaglobulinemia suggested by the history.

After May 1957 the patient was followed up as an outpatient. Serum protein electrophoresis repeatedly demonstrated a gamma globulin level between 0.4 and 0.45 gm. per 100 ml. (Table III.) Results of complement fixation tests for histoplasmosis and blastomycosis were negative. Numerous cultures of sputum and exudate from the ulcers of the leg were negative for tubercle bacilli and fungi. The antistreptolysin O titer was less than 6 units; the isohemagglutin titer was 1:2; and a sheep cell agglutination test for rheumatoid arthritis was negative. Challenge with 0.5 cc. typhoid vaccine raised the Widal titers from 0 to 1:80 for "H" and 1:40 for "O" antigens.

During June and July 1957 the uptake and die-

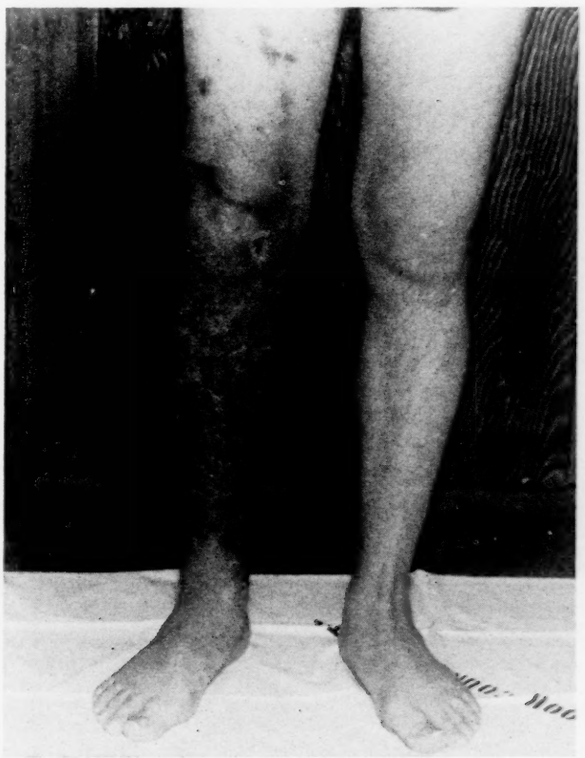


FIG. 1. Photograph of proband (Case 1) showing ulcerations of right leg.

TABLE III

PROTEIN ELECTROPHORETIC PARTITION OF HYPOGAMMAGLOBULINEMIC AND NORMAL MEMBERS OF THE FAMILY*

Case No.	Relationship to Proband	Age (yr.)	Serum Proteins (gm./100 ml.)					
			Total Protein	Albumin	Alpha-1 Globulin	Alpha-2 Globulin	Beta-Globulin	Gamma Globulin
Patients with Hypogammaglobulinemia								
1	Proband	42	6.0	**3.3**	**0.54**	**0.72**	**1.02**	**0.43**
			5.9	*3.1*	*0.70*	*0.90*	*0.76*	*0.42*
2	Son	14	6.4	**4.1**	**0.45**	**0.58**	**0.83**	**0.45**
			6.4	*3.4*	*0.84*	*0.84*	*0.79*	*0.47*
3	Daughter	6	6.1	**4.1**	**0.37**	**0.61**	**0.61**	**0.37**
			6.1	*3.5*	*0.49*	*0.91*	*0.67*	*0.45*
4	Daughter	6	6.5	**4.7**	**0.26**	**0.52**	**0.59**	**0.39**
			6.5	*4.0*	*0.62*	*0.78*	*0.73*	*0.39*
Normal Subjects								
5	Wife	39	6.8	3.4	0.47	0.54	0.81	1.56
6	Son	15	7.2	4.2	0.36	0.72	0.72	1.15
7	Daughter	10	7.5	4.4	0.37	0.67	0.82	1.27
8	Daughter	8	7.0	4.3	0.28	0.56	0.77	1.05
9	Father	75	7.7	4.8	0.46	0.69	0.62	1.16
10	Mother	73	7.3	4.5	0.36	0.73	0.80	0.94
11	Brother	46	8.4	5.2	0.33	0.75	0.92	1.09
12	Sister	40	7.3	4.4	0.32	0.69	0.76	1.10

NOTE: Italicized figures from moving boundary electrophoresis (Tiselius-American Instrument Co.). Bold faced figures from paper electrophoresis (Spinco).

away plots of S^{35}-methionine incorporation into the serum proteins were investigated. Uptake of isotope into gamma globulins, a reflection of synthesis rate, was markedly depressed. Uptakes into albumin, and the other globulin fractions were normal. The apparent biological turnover time of the small amount of gamma globulins formed was markedly decreased, either because of failure to reutilize the S^{35} label or as a result of abnormally rapid degradation. The apparent turnover time for albumin was normal. Following this the patient was given a loading dose of 55 gm. of pooled Red Cross gamma globulin, intramuscularly. His serum gamma globulin level rose to 0.67 gm. per 100 ml. The subsequent die-away plot showed an apparent turnover time greatly prolonged over that obtained in normal subjects and patients with idiopathic hypogammaglobulinemia, but this may have been due to faulty procedure. These observations have been reported in detail elsewhere [*3*]. Their significance will be discussed subsequently.

Following completion of these studies the patient was maintained on monthly injections of 6.6 gm. of gamma globulin. On this regimen his serum gamma globulin level was unchanged. Subjectively, however, the patient claimed great relief particularly from symptoms of chronic fatigue. Objectively, marked healing of the ulcers of the leg was observed.

In October 1957, while receiving gamma globulin, the patient again had chills, fever and a cough which did not respond to antibiotic therapy. After six weeks, diarrhea, vomiting, dehydration and prostration necessitated admission to a local hospital. Physical examination revealed only the recurrence of extensive ulcers of the right leg.

Urinalysis showed proteinuria and occasional casts. Chest roentgenograms were non-contributory. Cultures of the stool and urine were negative. A biopsy specimen of an ulcer of the leg showed necrosis, hyalinized tissues and infiltration by polymorphonuclear leukocytes. Hematologic determinations are shown in Table I. When his condition was unchanged after five days of erythromycin and chloramphenicol infusion (2 gm. each daily), 23 gm. of gamma globulin was administered intramuscularly in four days. This seemed to effect a remarkable and rapid recovery, and within two weeks he was well enough to return to work.

Early in 1958, however, sinusitis, chronic cough and

fatigue recurred. In March he elected to spend the summer in Arizona "for his health." In May 1958 he saw a physician because of cough. A roentgenogram of the chest now revealed a fine reticular miliary infiltration throughout the lungs, but most pronounced on the left. Cultures of the sputum were negative. Results of skin tests with coccidioidin and purified protein derivative No. 2 were negative, while those of histoplasmin were again positive. The patient was treated with a variety of drugs including penicillin, streptomycin, hydrocortisone, decongestives and antihistaminics. He also continued to receive monthly injections of gamma globulin.

The patient returned to Chicago in July 1958. He had lost 55 pounds, appeared weak and was slightly dyspneic at rest. Slight clubbing of the fingernails was observed for the first time. The chest was clear to auscultation and percussion. The ulcers of the leg were almost completely healed. Results of complement fixation tests for histoplasmosis and blastomycosis were negative. The erythrocyte sedimentation rate was 5 mm. per hour. Hematologic and biochemical determinations are shown in Tables I and II.

A presumptive diagnosis of miliary tuberculosis was made. He was urged to seek hospitalization, and antituberculosis therapy was instituted. However, he remained at home where he pursued a rapid downhill course. He died on August 2, 1958, after four years of chronic ill health. A sputum culture obtained fourteen days prior to death grew Histoplasma capsulatum on the twenty-seventh day.

At necropsy all lymph nodes examined showed uniform absence of lymphoid follicles and a general paucity of lymphoid elements. There was proliferation of the sinusoidal reticuloendothelial cells but an almost complete absence of plasma cells. The spleen was enlarged (1,020 gm.), and showed hyperplasia of the reticulum cells with almost complete absence of plasma cells. In the middle lobe of the right lung there was a large, calcified, round lesion which measured 1.5 by 1.5 cm. Similar calcifications were present in the lower right and left lobes, many with a laminated appearance. Bronchopulmonary lymph nodes also were calcified. Scattered throughout the entire pulmonary parenchyma, but most numerous in the upper lobes, were small, gray-white nodules 1 to 4 mm. in diameter. Similar tubercle-like lesions were scattered throughout the spleen, liver, lymph nodes and bone marrow. Microscopically, these consisted of epithelioid cells and Langhans' type of multinucleated giant cells, and both hematoxylin and eosin and periodic acid-Schiff stains revealed numerous organisms morphologically resembling Histoplasma capsulatum. Both adrenal glands were enlarged (40 gm.). Extensive caseation necrosis had destroyed the greater part of the cortex and medulla but some cortical tissue was intact. Microscopically, there was a massive caseation necrosis with epithelioid cells and Langhans' type of multinucleated giant cells. Again, numerous intracellular and extracellular organisms resembling histoplasma were present. Despite the severe involvement of the various organs, the number of phagocytosed histoplasma organisms was not as great as ordinarily seen in systemic histoplasmosis. Subsequently, Histoplasma capsulatum was isolated in pure culture from the various lesions. All cultures for tuberculosis were negative. The skin of the right leg showed atrophy and superficial ulcerations. No vascular changes were found and cultures for histoplasma were negative. The media of the aorta showed a diffuse decrease of elastica while the adventitia and the vasa vasorum appeared normal. The kidneys showed a few pyelonephritic scars. The other organs were normal. The final pathological diagnoses were generalized systemic histoplasmosis involving the lungs, liver, spleen, adrenals, lymph nodes and bone marrow; idiopathic adult hypogammaglobulinemia; pulmonary emphysema; bilateral pleural adhesions; fatty liver; and chronic pyelonephritis. Additional details of the necropsy findings have been presented elsewhere [*4*].

CASE II. A fourteen year old boy, the proband's second child, has had frequent recurrent infections of the upper respiratory tract and fevers of obscure origin since early childhood. He had measles, mumps and varicella between the ages of four and six. He often complains of intermittent pain without swelling of the right knee. When ten years old, leukopenia was demonstrated (Table I) at the time it was first found in his father. Physical examination revealed a well developed and well nourished adolescent boy. Slight lymphadenopathy was present. A sharp-edged spleen was readily palpable 4 cm. below the left costal margin. The right knee appeared normal. Roentgenograms revealed an increase in pulmonary bronchial markings, an enlarged spleen and a spina bifida occulta of the first sacral vertebrae. The isohemagglutinin titer was 1:16, the antistreptolysin O titer less than 12 units; the typhoid agglutination titers were negative. Results of the Schick test as well as the Mantoux, histoplasmin skin and complement fixation tests were negative. Serum protein electrophoresis demonstrated hypogammaglobulinemia. (Table III.) Bone marrow aspiration showed an occasional plasma cell and findings suggestive of a hemolytic process. Bone marrow cultures were negative for fungi. The results of additional laboratory investigations are listed in Tables I through III.

CASES III AND IV. Six year old twin girls, the proband's youngest children, have had frequent, recurrent, infection of the upper respiratory tract and fever of obscure origin since early childhood. Both had measles when three years old. Physical examination revealed two well developed, slender young girls. Lymphadenopathy, usually expected at this age, was absent. A sharp-edged spleen extending 3 cm. below

the left costal margin was readily palpable in both. Roentgenograms revealed increased pulmonary bronchial markings, enlarged spleen, and occult spina bifida of the first sacral vertebrae. Laboratory investigations demonstrated hypogammaglobulinemia and leukopenia of the same degree present in their father and brother. (Tables I through III.) The fraternal relationship of the twins is indicated by a history of separate placentas at birth and by demonstration of a difference in blood Rh genotype. The initial isohemagglutin titers in the twins were 1:200 in each. Investigation revealed that both had received booster doses of triple vaccine (diphtheria, tetanus and pertussis) six weeks prior to testing. Repeat titers 3 months later were 1:16. Results of the Schick tests were negative, as were the Mantoux, histoplasmin skin and complement fixation tests. The antistreptolysin O titer was less than 12 units and the typhoid agglutination titers were negative. Bone marrow aspirations revealed absence of plasma cells in one and an occasional plasma cell in the other twin. Bone marrow cultures for fungi were negative.

Further Familial Investigations. None of the other members of the family have a history of undue susceptibility to infection. The proband's seventh child, a daughter and third in chronological order, died at the age of two and one half months. The autopsy records revealed multiple osseous abnormalities including hydrocephalus, lacunar skull, spina bifida (through the twelfth thoracic and lumbar vertebrae), unilateral talipes varus and suspect osteogenesis imperfecta congenita. Physical examination of the proband's father revealed a barely palpable liver and spleen. His serum gamma globulin level and white blood cell count were normal. (Tables I and III.) Roentgenograms did not reveal any pertinent abnormalities. There was no history of increased susceptibility to infection in either his or his wife's parents and siblings. Physical and laboratory examination of the proband's wife, his three other living children, his mother, his brother and his sister revealed no hypogammaglobulinemia, splenomegaly or leukopenia. (Tables I, II and III.) Two of the normal daughters have a spina bifida occulta of the first sacral vertebra. The blood count of the proband's brother revealed an unexpected polycythemia. It has not been possible to investigate this further. The results of the Mantoux test of the proband's wife were positive. Results of the Mantoux and skin and complement fixation tests for histoplasmosis were otherwise negative in the immediate family. The isohemagglutinin titers in the normal members of the family ranged from 1:32 to 1:64, the antistreptolysin O titers from 12 to 50 units. An interesting biochemical finding in the involved family members was hyperuricemia. (Table II.) The only members of the family who were not examined were the proband's two nieces, neither of whom reportedly have undue susceptibility to infection. There is no consanguinity within the family.

REVIEW OF THE ETIOLOGICAL FACTORS OF THE HYPOGAMMAGLOBULINEMIAS

The newborn child lacks plasma cells in its bone marrow and lymph nodes, is unable to form gamma globulin, and is not capable of adequate antibody production in response to antigenic stimulation [*5*]. The child at birth is therefore essentially an agammaglobulinemic individual [*5,6*] who is protected from the consequence of this defect by the passive placental transfer of maternal gamma globulins. The levels of maternally derived serum gamma globulins and specific antibodies decline exponentially and virtually reach zero by the fifth or sixth month of life [*2,5,6*]. The lymph nodes at birth also show poor cortical development and absent secondary germinal follicles. They resemble those observed in congenital agammaglobulinemia [*7*]. During the second month of life, differentiated plasma cells appear in the infant's lymph nodes and bone marrow. The production of gamma globulin begins and weak antibody responses to antigenic stimulation become evident [*5,8*]. Secondary germinal follicles appear in the lymph nodes [*5*]. Adequate levels of gamma globulin are approached by the ninth month of life, but adult levels may not be achieved until the seventh or eighth year [*9*]. In the second and third months of life, during the period when maternal gamma globulin is declining and the formation of gamma globulin by the infant is as yet inadequate, a transient period of hypogammaglobulinemia develops with levels of 300 to 600 mg./100 ml. [*9–11*]. If the newborn is initially inadequately provided with maternal gamma globulins or if the time of onset of gamma globulin formation is delayed, the level of gamma globulins and antibodies at this time may be inadequate to protect the child from bacterial infection, and replacement therapy may be necessary. Clearly, the diagnosis of agammaglobulinemia should neither be made at this time nor until the child is more than nine months old. Inability to form gamma globulin in the guinea pig fetus [*12*] and to form gamma globulin and produce antibodies in the newborn rabbit [*5,13*] has also been described and may well be universal among mammals. The physiological function subserved by this initial retardation of plasma cell differentiation and failure of gamma globulin and antibody formation is not clear. Its elucidation will undoubtedly greatly enhance our understanding of the immune process [*5*].

In the entity of congenital agammaglobulinemia, small amounts of gamma globulins, 4 to 50 mg./100 ml., can with rare exceptions be measured by immunochemical procedures [*6,14*]. This amount of gamma globulin is too small to be observed upon zone electrophoresis, hence the term "agammaglobulinemia." The onset of increased susceptibility to infection can often be dated to about the sixth month of life at which time the protecting properties of the maternal gamma globulins have vanished. The illness occurs only in boys [*6,14*] and the affected individual's brothers, maternal uncles and maternal male cousins may also have the same condition [*6*]. The bone marrow and lymph nodes of these patients lack plasma cells, and intensive antigenic stimulation fails to produce specific antibody formation [*14*]. Histologically, the lymph nodes also show a thin cortex, a decrease in the number of primary follicles and absence of secondary germinal follicles [*14*]. When the turnover time of exogenous gamma globulins is measured in these patients, a half life of about thirty days is obtained [*7,14*]. In normal subjects similarly obtained half lifes usually measure twenty days [*15*]. Hematologic disturbances, particularly leukopenia, but also hemolytic anemia and lymphopenia, are often observed [*14*]. Splenomegaly, if it occurs at all, is unusual [*6,14,16,17*]. The evidence indicates that this disease is due to hereditary transmission of a sex-linked recessive gene. It is theoretically possible, but statistically unlikely, that the disease may appear in girls [*6*]. This genetic defect leads presumably to failure of plasma cell differentiation with consequent inability to form gamma globulin and produce antibodies. The somewhat prolonged turnover time for exogenous gamma globulins in these patients has been accepted as evidence that the low levels are not due to its increased destruction or loss [*7,14,18*]. It is not clear why the half life of gamma globulins in these patients should be longer than in normal subjects.

In the syndrome of idiopathic hypogammaglobulinemia the level of gamma globulins may range from that observed in congenital agammaglobulinemia to 350 mg./100 ml. [*6*]. The onset of disease usually occurs during adult life, and some cases therefore have been designated "adult" idiopathic hypogammaglobulinemia. However, a case has been described in a child three years old with a history of recurrent infections from the age of eight months [*19*]. The disease occurs in both sexes. It has not, to our knowledge, been reported in more than one member of a single family. In some cases, however, hypergammaglobulinemia has been found in both parents of the affected individual [*20,21*]. Examination of the bone marrow and lymph nodes reveals either absence or marked decrease in the number of plasma cells. The histological appearance of the lymph nodes may resemble that seen in congenital agammaglobulinemia, but both replacement of the normal architecture by a granulomatous process and benign follicular hyperplasia have occasionally been observed. Upon antigenic stimulation either no antibody response occurs or it is markedly reduced. The turnover time for exogenously administered gamma globulin is similar to that observed in congenital agammaglobulinemia, that is, it is somewhat prolonged when compared to normal subjects. These patients may exhibit the same hematological disorders observed in congenital agammaglobulinemia. Splenomegaly may be absent but more often it is present and is sometimes accompanied by hepatomegaly. This syndrome has often been designated "acquired" idiopathic hypogammaglobulinemia upon the assumption that its usual late onset in life indicates failure of a previously intact, functioning immune system. Gitlin and Janeway [*6*], however, have suggested that the same sequence of events may occur from an hereditary disturbance which manifests itself late in life. Zelman and Lewin [*21*] have suggested that the observed parental hypergammaglobulinemia may possibly represent the effect of a recessive gene for hypogammaglobulinemia, the latter appearing only in a double recessive inheritance. Whatever the mechanism, there is failure of the plasma cell to survive, and the ability to form gamma globulins and produce antibodies either vanishes or becomes depressed to a point where the individual is unable adequately to resist bacterial infection. As in congenital agammaglobulinemia, the prolonged turnover time observed for exogenous gamma globulins in these patients has been accepted as evidence that the low levels are not due to its increased destruction or loss. A markedly decreased ability to form gamma globulins has been demonstrated in two patients with idiopathic hypogammaglobulinemia and in the proband of this study by direct isotopic measurement of protein synthesis rates [*3*]. In one of these patients and in the proband

the small amounts of gamma globulin formed showed an abnormally rapid turnover rate. This contrasted strikingly to prolonged turnover rates obtained in both patients with exogenous gamma globulin. The cause of this discrepancy is unknown but it is clear that the manner in which an individual may metabolize exogenous gamma globulins does not necessarily correspond to his handling of endogenously formed gamma globulin [*3*].

Secondary hypogammaglobulinemia has multiple etiologies and in any one category more than one factor may play a role. Diffuse diseases of the reticuloendothelial system may be associated with hypogammaglobulinemia. In multiple myeloma, it is probable that the abnormal plasma cells producing an abnormal globulin may replace normal plasma cells, with a consequent fall in the level of normal gamma globulins and decrease in ability to produce antibodies [*22*]. Recent studies [*3,23*] have suggested that the paradoxical hypogammaglobulinemia of myeloma is the result of a competition for amino acid substrates in which the system responsible for producing huge amounts of the distinctive myeloma globulin has priority over the system producing normal gamma globulins. The infrequent instances of hypogammaglobulinemia observed in patients with chronic lymphatic leukemia and lymphosarcoma are apparently due to replacement of the plasma cells [*24–27*]. It is not clear why hypogammaglobulinemia is observed only infrequently in these conditions and why, in some patients with lymphosarcoma, hyperglobulinemia is observed. Hypogammaglobulinemia may also be observed in conditions resulting in external losses of serum gamma globulin. In the nephrotic syndrome, some loss occurs through the kidneys. In addition, some displacement into edema fluid may occur, and Gitlin and Janeway [*28*] have produced evidence that an increase in the turnover rate of gamma globulins also contributes to the observed hypogammaglobulinemia. In burns there is oozing of gamma globulin from denuded skin surfaces but dilution of proteins from shifts in body water also contributes to the hypogammaglobulinemia sometimes observed [*29*]. A temporary hypogammaglobulinemia may also occur after severe hemorrhage. Agents which directly injure the reticuloendothelial system, such as radiation and nitrogen mustard, may also be a cause of hypogammaglobulinemia [*2*]. Finally, hypogammaglobulinemia may be observed in rare instances of hypercatabolic hypoproteinemia [*7*].

COMMENTS

The syndrome found in this family resembles closely the syndrome of hypogammaglobulinemia, splenomegaly and hypersplenism described and reviewed by Prasad, Reiner and Watson [*30*] who note that this syndrome is relatively common. However, to our knowledge, idiopathic hypogammaglobulinemia, with one possible exception (to be considered subsequently), has not been observed in more than one member of a family, although in many reports serum protein studies have been performed for the entire family. The family described differs in other respects from that of the usual patient with idiopathic hypogammaglobulinemia. The level of circulating gamma globulins is distinctly higher, the degree of immunologic responsiveness is correspondingly greater as indicated by the proband's response to typhoid antigen, the presence of a negative reaction to the Schick test in the affected children, and the non-specific isohemagglutinin response to triple vaccine injection in the twins. This latter response has been attributed to the tetanus toxoid component of the vaccine [*31*]. The presence of spina bifida occulta in both the affected and normal children, of hyperuricemia in the involved family members, and of polycythemia in the proband's brother are probably coincidental.

The peculiar ulcerations of the leg found in the proband have not been explained. Similar ulcerations, also sometimes accompanied by monoarticular arthritis, have been described in idiopathic hypogammaglobulinemia [*22,32*]. Homberger and Petermann [*33*] have described a syndrome, "familial idiopathic dysproteinemia," characterized by hypoproteinemia or abnormalities of the plasma electrophoretic pattern, ulcers of the legs in adult men, low oscillometer indices in adult women and edema. Two adults, a man and a woman, had hypogammaglobulinemia. Splenomegaly was not reported. The white blood counts were at the lower normal range. Muscle biopsy specimens showed normal blood vessels. An injection of gamma globulin in the woman with hypogammaglobulinemia was observed to disappear at an abnormally rapid rate. This interesting report clearly described the entity of idiopathic hypogammaglobulinemia several years before

Bruton's original communication. The presence of splenomegaly and leukopenia, and absence of hypoproteinemia and edema, distinguishes our family from theirs.

The entity presented by this family can be readily differentiated from congenital agammaglobulinemia for it occurs in both sexes, splenomegaly is present, the serum gamma globulins level is higher, and the degree of immunologic responsiveness is greater than found in that condition. The possibility that the hypogammaglobulinemia is secondary to some other disease seems unlikely, for with the exception of histoplasmosis in the proband, no other illnesses can be demonstrated. Histoplasmosis in a patient with idiopathic hypogammaglobulinemia has been reported once before and that patient survived [*34*]. There is also no history of common exposure to any other infectious or noxious agents. The history of the proband suggests that the hypogammaglobulinemia may well have been present since early childhood, antedating the birth of his children.

The distribution of the clinical features of this entity in an adult and half of his children of both sexes suggests the possibility of an hereditary disturbance transmitted by an autosomal dominant gene. The presence of hepatosplenomegaly in the proband's father could represent an example of incomplete penetrance by a dominant gene. However, in a study limited to one family, the possibility that the entity is a result of doubling of an abnormal, recessive gene when the proband is heterozygous cannot be excluded.

SUMMARY

The death from disseminated histoplasmosis of a patient with hypogammaglobulinemia, increased susceptibility to infection, splenomegaly and leukopenia led to an investigation of his family. Three of his six living children, a thirteen year old boy and six year old fraternal twin girls, were found to have a similar syndrome without evidence of infection with histoplasmosis. The patient's father had splenomegaly but no other features of this syndrome. The other members of the family had none of these abnormalities. In the involved members the level of gamma globulins and the degree of immunologic responsiveness, while depressed, were greater than usually observed in other forms of hypogammaglobulinemia. The evidence suggests that this apparently new clinical entity is an hereditary disturbance transmitted possibly by an autosomal dominant gene.

The etiological features of the known forms of hypogammaglobulinemia are reviewed and commented upon.

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Serum Alkaline Phosphatase Activity in Hepatitis of Infectious Mononucleosis*

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MARKED elevations of serum alkaline phosphatase activity are recognized as a common finding in cases of extrahepatic obstruction of the biliary tree, and clinical studies have borne out the usefulness of this determination in the differential diagnosis of jaundice of intrahepatic and extrahepatic origin [*1–3*]. In recent years, however, clinical reports have indicated that striking increases in serum alkaline phosphatase activity may accompany a variety of intrahepatic lesions, including so-called cholangiolitic viral hepatitis [*4,5*], jaundice due to administration of methyltestosterone [*6,7*] and chlorpromazine [*8,9*], hepatic gumma [*10*] and tuberculosis [*11*], primary [*12*] and metastatic [*13–15*] malignant disease of the liver, primary biliary cirrhosis [*16*], sarcoidosis [*17*], amyloidosis [*17*], disseminated lupus erythematosus [*17*], postnecrotic cirrhosis [*18*] and jaundice due to administration of chlorothiazide [*19*]. In most of these latter instances a striking disproportion between the magnitude of the elevations of serum alkaline phosphatase activity and the relatively minor increases in serum bilirubin concentration has been noted.

Experience at The New York Hospital in the past few years has indicated that infectious mononucleosis should be included among the causes of this pattern of hepatic dysfunction; this experience is presented herewith.

MATERIAL AND METHODS

All charts in the Record Room of The New York Hospital indexed as cases of infectious mononucleosis and occurring since January 1, 1950, were reviewed. Those not showing typical clinical and hematological features of the disease were discarded. The required clinical features included most or all of the following: fever, pharyngitis, cervical or generalized lymphadenopathy, hepatomegaly and/or splenomegaly. Hematological features required for inclusion in the study were lymphocytosis of 50 per cent or more, the presence of abnormal lymphocytes of the type described by Downey [*20*] in infectious mononucleosis, and titers of heterophil agglutinins of 1:896 or more; in the case of titers lower than this, differential absorption studies with guinea pig kidney and beef erythrocytes were required to be diagnostic of infectious mononucleosis.

The various hematological, serological and chemical studies were made in the routine laboratories of the hospital. Normal values in these laboratories are indicated in Table I.

Of 123 cases meeting the criteria employed, in seventy-three chemical investigations of liver function, including determinations of serum alkaline phosphatase activity, had been carried out during the patient's course in the hospital. This report is based on these cases.

RESULTS

In sixty-four patients serum alkaline phosphatase activity was elevated at some time during the course of the illness. The peak values observed are summarized in Table II.

It will be seen that marked elevations occurred in ten patients, comprising 13.7 per cent of the group in which liver function tests were carried out. A more detailed presentation of data on representative cases in this group of patients follows.

CASE REPORTS

CASE I. A twenty-nine year old white man with diabetes (NYH No. 563478) entered the hospital on February 20, 1950. Headache had been noted for two weeks, and for three days there had been nausea, vomiting, epigastric discomfort and dark urine. Jaundice appeared the day before admission. Examination revealed low grade fever, slight icterus, pharyngeal injection and generalized lymphadenopathy. There was slight hepatosplenomegaly. The urine contained no bile; there were 11 Ehrlich units of urobilinogen in 500 cc. Sternal marrow aspiration on

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TABLE I
NORMAL VALUES FOR VARIOUS PROCEDURES AT THE NEW YORK HOSPITAL

Serum bilirubin (mg. %)	
Total	0.1–0.8
Direct (1 minute)	0–0.2
Indirect	0.1–0.6
Serum alkaline phosphatase activity (Bodansky units)	4 or less
Thymol turbidity (units)	Up to 4
Cephalin-cholesterol flocculation (units)	Up to 4
Total serum proteins (gm. %)	6.0–8.0
Albumin	4.0–5.2
Globulin	2.0–2.8
Bromsulphalein retention (5 mg./kilo injected)	5% retention after 45 minutes
Serum glutamic oxalacetic transaminase (units)	Up to 40
Total serum cholesterol (mg. %)	150–250
Cholesterol esters (%)	65–75

the third hospital day revealed that 59 per cent of the leukocytic forms present were lymphocytes, of which half were Downey I cells.

Pertinent laboratory data are summarized in Table III.

Comment: The disproportion between serum alkaline phosphatase activity and the serum bilirubin became more striking under observation. The titer of heterophil agglutinins, the peripheral blood lymphocytosis and bromsulphalein retention all fell toward normal as the serum alkaline phosphatase activity rose. Marked abnormality of the cephalin-cholesterol flocculation reaction accompanied the other alterations of liver function.

CASE II. A twenty-two year old female laboratory technician (NYH No. 544084) entered the hospital on March 1, 1950. She had noted malaise for five days, and in the four days before admission had had shaking chills, fever, sore throat and cervical adenitis. On examination there was no icterus. The pharynx was diffusely injected, and there was cervical and axillary lymphadenopathy. Slight hepatosplenomegaly was noted. The urine was free of bile.

In the first week in the hospital the liver and spleen increased in size; thereafter they receded.

The laboratory studies are summarized in Table III.

TABLE II
PEAK VALUES OF SERUM ALKALINE PHOSPHATASE ACTIVITY IN SEVENTY-THREE CASES OF INFECTIOUS MONONUCLEOSIS

Alkaline Phosphatase (Bodansky units)	Cases	
	No.	Per cent
4 or less	9	12.3
4.1–10.0	33	45.2
10.1–15.0	14	19.2
15.1–20.0	7	9.6
Over 20	10	13.7
Totals	73	100.0

Comment: An elevation in serum alkaline phosphatase activity occurred, with no abnormality in serum total bilirubin; the peak level of alkaline phosphatase activity correlated in time with the appearance of hyperglobulinemia, but not with peak titers of heterophil agglutinins or peripheral blood lymphocytosis. Moderate abnormalities in the flocculation tests were noted.

CASE III. A twenty-two year old white woman (NYH No. 233217) was admitted to the hospital on November 12, 1954. Two weeks prior to admission she had had anorexia, loss of taste for cigarettes, and yellowing of the skin. The stools were light and urine dark in the two to three days before admission. There had been no sore throat or pruritus. On examination the temperature was 38.3°C. rectally, with a concomitant tachycardia. There was obvious icterus, an ulcerated lesion on the left anterior tonsillar pillar, slightly tender hepatosplenomegaly and generalized lymphadenopathy.

Fever persisted for five days. Her course was marked also by electrocardiographic changes compatible with acute myocarditis.

The laboratory studies are indicated in Table IV.

Comment: Slight hyperglobulinemia coexisted with marked elevation of serum alkaline phosphatase activity. Jaundice was present on admission, and the serum bilirubin, which fell after admission, rose again transiently without an associated rise in alkaline phosphatase activity.

CASE IV. A nineteen year old white female student nurse (NYH No. 666113) was admitted to the hospital

TABLE III

Date (1950)	White Blood Cells (per cu. mm.)	Lymphocytes / Atypical Lymphocytes (%)	Bilirubin (mg. %) Total	Direct	Indirect	Thymol Turbidity (units)	Cephalin Flocculation (units)	Alkaline Phosphatase (Bodansky (units)	Bromsulphalein*	Albumin/ Globulin (gm. %)	Heterophil Agglutinins
					Case I						
2/20	12,200	59 / 17	3.6	2.6	1.0	6	..	17.8	40	3.8/2.4	1:3584
2/21	22,000	69 / 61	...	...	...	..	19	14.8	....	.....	.....
2/27	8,300	51 / ..	2.0	1.3	0.7	..	17	26.1	22	.....	1:896
3/20	.....	.....	0.8	...	...	..	7	9.9	....	.....	.....
					Case II						
3/2	5,300	64 / "nearly all"	...	...	...	..	..	....	35	.....	1:1792
3/4	9,000	78 / 39	...	...	...	3	..	3	....	.....	.....
3/8	.....	.....	...	...	...	..	..	....	39.9	.....	.....
3/9	.....	.....	1.1	...	...	9	16	....	....	3.9/2.9	1:896
3/15	19,250	.....	...	...	...	..	..	....	....	.....	.....
3/16	.....	.....	0.6	...	...	8	..	20.1	17.1	4.2/3.4	.....
3/23	.....	.....	0.8	..	...	..	..	10.9	23	.....	.....
3/31	.....	.....	1.1	...	...	6	10	5.5	15	4.8/3.2	.....
4/3	6,400	58 / ..	...	...	...	..	..	....	....	.....	1:224
4/7	.....	.....	0.5	...	...	5	1	4.4	1.3	5.0/2.9	.....

* Per cent bromsulphalein retention after forty-five minutes; 5 mg./kilo injected.

TABLE IV

CASE III

Date	White Blood Cells (per cu. mm.)	Lymphocytes / Atypical Lymphocytes (%)	Alkaline Phosphatase (Bodansky units)	Bilirubin (mg. %) Total	Direct	Indirect	Thymol Turbidity (units)	Cephalin Flocculation (units)	Bromsulphalein*	Cholesterol / Esters (%)	Total Protein	Albumin/ Globulin (gm. %)	Heterophil Agglutinins
11/12/54	24.1	90 / 80	....	...	...	...	..	..	...	...	...	.....	.....
11/13/54	....	..	33.2	7.2	4.4	2.8	11	..	...	134 / 49	7.4	4.3/3.1	1:7168
11/19/54	....	..	....	...	...	...	..	..	...	...	...	.....	1:3584 1:448† 1:14‡
11/22/54	11.8	78 / 47	19.3	1.2	0.6	0.6	8	6	...	...	...	.....	.....
12/3/54	7.2	..	12.7	3.6	2.1	1.5	..	..	...	...	7.2	4.7/2.5	.....
12/15/54	....	..	4.8	0.8	...	...	6	..	1.9	...	...	.....	.....
12/20/54	....	..	4.7	0.5	0.3	0.2	5	0	...	...	...	.....	.....
4/5/55	....	..	....	0.1	...	...	4	5	...	...	...	.....	.....

* Per cent bromsulphalein retention after forty-five minutes; 5 mg./kilo injected.
† After guinea pig kidney absorption.
‡ After beef red blood cell absorption.

on November 23, 1954, complaining of chilly sensations for three days and fever for twenty-four hours. On examination the temperature was 40°c., and there was slight relative bradycardia. She appeared acutely ill. The oropharyngeal mucous membranes were slightly injected, and there was slight bilateral cervical adenopathy. The spleen was felt 3 cm. below the left costal margin and was firm. The liver was not felt.

This patient had a long and stormy hospital course, marked by bilateral upper quadrant abdominal pain for a week after admission, the development of frank hepatitis and hemolytic anemia, and fever for five

TABLE V
CASE IV

Date	White Blood Cells (per cu. mm.)	Lymphocytes / Atypical Lymphocytes (%)	Hematocrit (%)	Reticulocytes (%)	Coombs' Test	Alkaline Phosphatase (Bodansky units)	Bilirubin (mg. %)			Thymol Turbidity (units)	Cephalin Flocculation (units)	Cholesterol / Esters (%)	Total Protein	Albumin/Globulin (gm. %)	Heterophil Agglutinins
							Total	Direct	Indirect						
11/23/54	5,000	17/15	44	...	.....	....	...	...	...	..	..	....	...	.....	.....
11/24/54	3,500	36/10	..	...	.....	2.1	0.5	0.1	0.4	6	9	....	...	.....	112
12/1/54	.....	..	..	...	.....	10.8	...	...	...	9	..	....	6.1	3.8/2.3	112
12/2/54	.....	..	38	1.4	.....	....	1.6	0.8	0.8	..	5	....	...	.....	1792
12/3/54	.....	..	34 36	...	Negative	12.7	3.6	2.1	1.5	12	14	....	6.2	3.2/3.0	448* .....
12/8/54	11,200	58 ..	35	4.7 6.5	.....	....	...	...	...	..	..	117 58.1	...	.....	.....
12/15/54	.....	..	..	...	.....	16.6	1.6	...	...	14	11	....	7.4	3.5/3.9	.....
12/17/54	.....	..	..	...	.....	21.3	1.8	...	...	11	17	....	7.9	4.1/3.8	.....
12/20/54	.....	..	38	...	.....	14.4	1.4	...	...	16	7	....	6.9	4.1/2.8	896
12/29/54	.....	..	..	...	.....	13.4	0.9	0.5	0.4	9	8	....	...	.....	.....
1/10/55	.....	..	..	2.0	Negative	6.3	0.9	0.3	0.6	9	7	....	...	.....	.....
1/14/55	.....	..	..	...	.....	5.6	0.6	0.4	0.2	9	..	....	...	.....	.....
2/7/55*	.....	..	..	...	.....	4.0	...	...	...	7	7	....	...	.....	.....
3/1/55	11,800	35	..	0.2	.....	....	0.5	0.1	0.4	..	..	....	...	.....	.....

* After guinea pig kidney absorption 1: 224; after beef red blood cell absorption 1: 14.

TABLE VI

CASE V

Date (1957)	White Blood Cells (per cu. mm.)	Lymphocytes / Atypical Lymphocytes (%)	Alkaline Phosphatase (Bodansky units)	Bilirubin (mg. %)			Thymol Turbidity (units)	Cephalin Flocculation (units)	Bromsulphalein*	Cholesterol / Esters (%)	Total Protein	Albumin/Globulin (gm. %)	Transaminase	Heterophil Agglutinins
				Total	Direct	Indirect								
2/28	9,300	56	26.8	1.0	0.4	0.6	11	14	19.3	211/62	7.3	4.9/2.4	113	3584
3/2	.....	..	15.1	0.7	0.3	0.4	..	..	....	....	...	.....	...	.....
3/4	14,000	64	9.8	0.6	...	...	..	7	14.6	....	...	.....	43	1792
3/7	.....	..	....	...	...	...	..	..	....	....	...	.....	...	448†
3/14	9,400	48	6.5	...	...	...	12	..	....	....	...	.....	...	896
7/5‡	.....	..	3.0	...	...	...	4	..	....	....	...	.....	...	.....

* Per cent bromsulphalein retention after forty-five minutes; 5 mg./kilo injected.
† After guinea pig kidney absorption 1:224; after beef red blood cell absorption 1:14.
‡ Follow-up.

weeks after admission. The pertinent laboratory studies are summarized in Table v.

Comment: This illness evolved rather slowly. Peak values for alkaline phosphatase activity appeared about one month after onset and were associated with marked abnormalities in the flocculation tests, but with eventual complete clearing of the chemical abnormalities of liver function. It is doubtful that the hemolytic anemia had any effect on the course of the hepatitis, since the anemia was mild and correlated poorly in time with the peak abnormalities of liver function. It is of interest to note the low normal value obtained for serum cholesterol at a time when the serum alkaline phosphatase activity was increased. In addition, concomitant fluctuations in serum globulin concentration and serum alkaline phosphatase activity are well demonstrated by this case.

CASE V. A twenty-eight year old white male lawyer (NYH No. 777159) was admitted to the hospital on February 27, 1957, with a history of twelve days of chilliness and diffuse myalgia, and one week of sore throat, marked malaise and dark urine. He had had an illness typical of acute infectious hepatitis in January 1955. In December 1955 the physical examination was within normal limits. Results of liver function tests at The New York Hospital were normal except for a thymol turbidity of 7 units.

At the time of this admission, the temperature was 37.9°C. and there was slight relative bradycardia. The patient was acutely ill. There was slight injection of the pharynx. The liver was not felt. The spleen was palpable 3 to 4 cm. below the left costal margin and was firm and non-tender.

The laboratory studies are summarized in Table VI.

Comment: The peak level of serum alkaline phosphatase activity occurred concomitantly with a normal serum globulin concentration. There was a slight increase in serum glutamic oxalacetic transaminase activity.

In general it may be stated that the patients described exhibited marked elevations in serum alkaline phosphatase activity without concomitant striking changes in serum bilirubin concentration, and that the levels of serum alkaline phosphatase activity fluctuated without necessary correlation in time with titers of heterophil antibody, with the proportion of lymphocytes in the peripheral blood, or with the size of the liver or spleen. In some cases there seemed to be an association in time between the level of serum alkaline phosphatase activity and the concentration of serum globulins, these values tending to rise and fall together.

In an effort to determine whether additional differences existed between the cases herein described and those in which lesser abnormalities of serum alkaline phosphatase activity were found, a comparison of the two groups of cases was made. The results are presented in Tables VII and VIII.

It is difficult to compare these groups closely in view of the difference in the numbers of patients in each group. By and large, the patients with marked elevation in serum alkaline phosphatase activity had slightly greater degrees of abnormality of several parameters which may be taken as indices of the severity of the illness; thus, this group had a slightly longer mean hospital stay than the group with only mild or moderate alterations of serum

TABLE VII

INFECTIOUS MONONUCLEOSIS WITH HEPATITIS

Comparison of Various Parameters in Patients with High and with Low Levels of Serum Alkaline Phosphatase Activity

Group*	Age (yr.)		Duration of Hospitalization (days)		Peak Fever (°C. rectal)		Duration of Fever (days)		Peak White Blood Cells (thousands per cu. mm.)		Peak Lymphocytosis (%)		Peak Titer Heterophil Agglutinins		Peak Serum Bilirubin (mg. %)		Peak Serum Globulin (gm. %)	
	Mean	Range	Mean	Range	Mean	Range	Mean	Range	Mean	Range	Mean	Range	Mean	Range	Mean	Range	Mean	Range
A	23.2	18–29	25.5	9–53	39.2	37.6–40.6	11.5	2–34	18.0	9.3–29.3	71.9	58–90	1:5018	1:1792–1:7168	2.9	0.9–7.2	3.04	2.4–3.9
B	22.9	15–41	19.4	2–68	38.9	37.8–40.7	9.3	0–30	12.9	4.8–27.7	75.2	41–93	1:4570	1:112–1:7168	1.2	0.1–12.1	2.72	1.6–4.2

* Group A = Peak level of serum alkaline phosphatase activity over 20 Bodansky Units.
Group B = Peak level of serum alkaline phosphatase activity 20 Bodansky Units or less.

TABLE VIII

COMPARISON OF VARIOUS PARAMETERS IN PATIENTS WITH HIGH AND WITH LOW LEVELS OF SERUM ALKALINE PHOSPHATASE ACTIVITY

Group*	Sex (%)		Hepatomegaly		Splenomegaly		Pharyngitis	
	Male	Female	No.	Per cent	No.	Per cent	No.	Per cent
A	40	60	7	70.0	9	90.0	7	70
B	48	52	41	64.1	45	70.3	48	75

* Group A = Peak level of serum alkaline phosphatase activity over 20 Bodansky Units.

Group B = Peak level of serum alkaline phosphatase activity 20 Bodansky Units or less.

alkaline phosphatase activity. In addition, the mean peak fever was slightly higher in the former group, the duration of fever was slightly greater, and the mean peak white blood cell count, mean heterophil agglutinin titer, mean serum bilirubin concentration and mean serum globulin concentration were all slightly higher. A greater proportion of the patients had splenomegaly. No striking difference between the groups is noted in terms of age or sex distribution of the cases, in the incidence of pharyngitis or hepatomegaly, or in the degree of peripheral blood lymphocytosis.

Some of the more marked differences between the two groups are examined in relation to levels of serum alkaline phosphatase activity in Figures 1 through 4. The quadrant test [*21*] has been used as an index of statistical significance in these figures, employing separation of the values by lines indicating the medians.*

By this technic the relationship between peak levels of serum alkaline phosphatase activity and peak titers of heterophil agglutinins does not achieve statistical significance. However, the relationship between peak serum alkaline phos-

* Correlations between the variables plotted in Figures 1 through 4 were evaluated by dividing the area of each graph by constructing its appropriate median lines. The total number of values falling in each of the resulting quadrants was determined. If the sum of the totals for the first and third quadrants (the first being that at the origin of the graph) is x_1, and the sum of the totals for the second and fourth quadrants is x_2, the following calculation can be made:

$$\frac{(x_1 - x_2)^2}{x_1 + x_2}$$

Values for this formula in excess of 3.84 indicate statistical significance at the 5 per cent level. (Derived from Mosteller [*21*].)

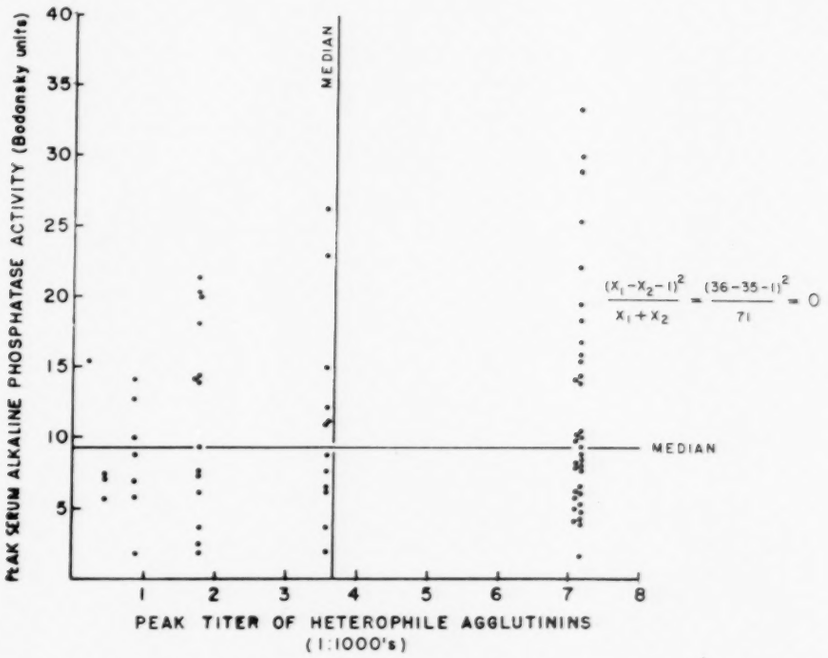


FIG. 1. Peak titer of heterophil agglutinins versus peak serum alkaline phosphatase activity (seventy-three cases).

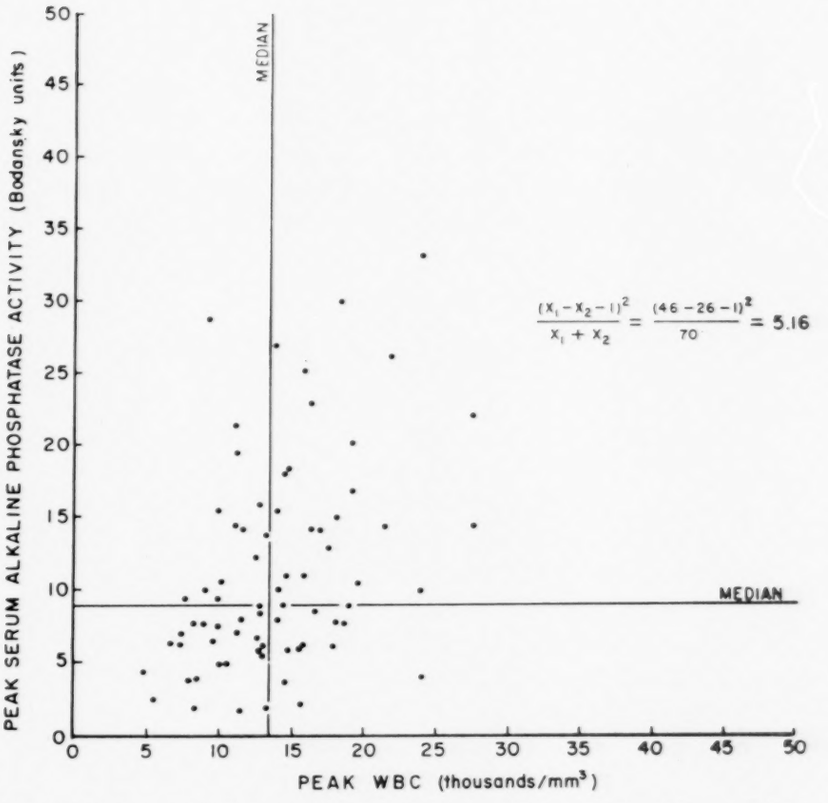


FIG. 2. Peak white blood cells versus peak serum alkaline phosphatase activity (seventy-three cases).

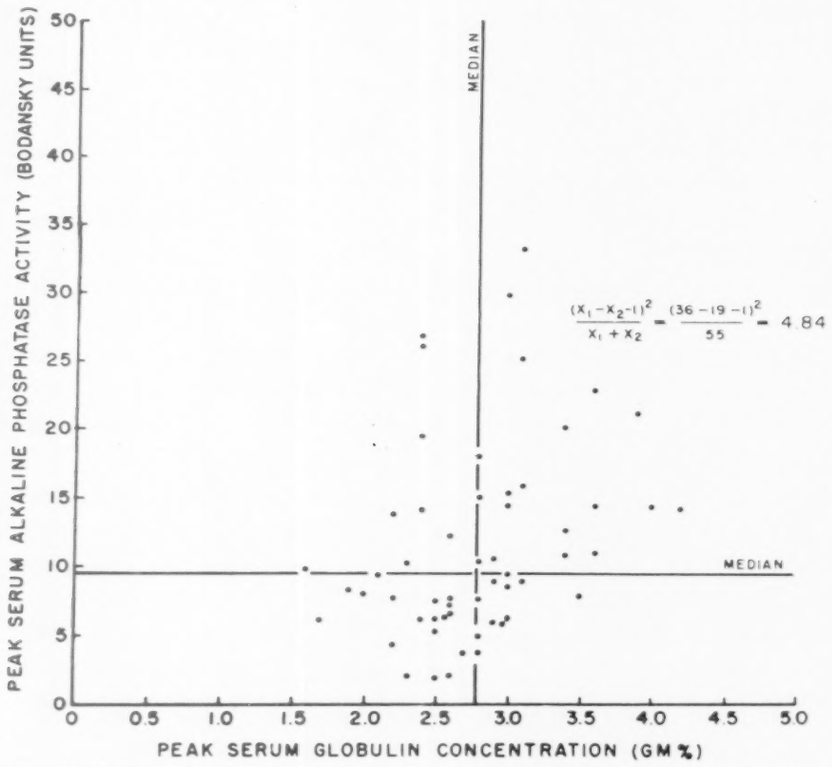


FIG. 3. Peak serum globulin concentration versus peak serum alkaline phosphatase activity (fifty-seven cases).

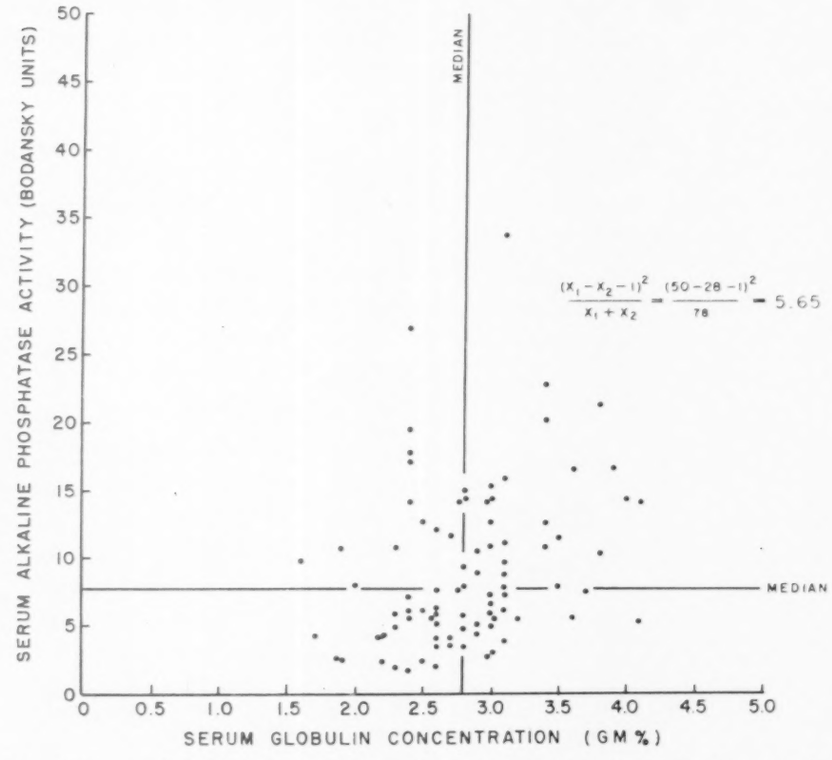


FIG. 4. Serum globulin concentration versus concomitantly determined serum alkaline phosphatase activity (forty-eight cases).

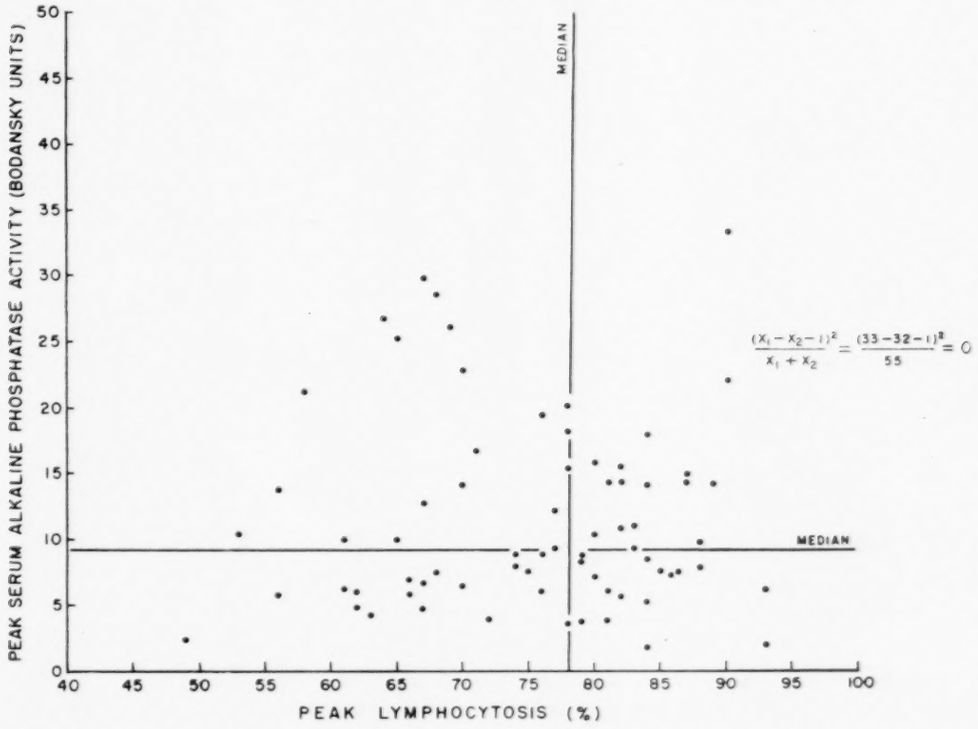


FIG. 5. Peak blood lymphocytosis versus peak serum alkaline phosphatase activity (seventy-one cases).

phatase activity and peak white blood cell counts does achieve statistical significance at the 5 per cent level. A statistically significant relationship at the 5 per cent level is also noted between serum alkaline phosphatase activity and serum globulin concentrations, and this is observed when peak values found during the course of the illness are plotted against each other as well as when concomitantly obtained values are plotted. Thus the data suggest that, although this relationship is not very striking, elevations of serum globulin concentration and moderate or marked elevations of serum alkaline phosphatase activity are likely to occur together during the acute phase of infectious mononucleosis with hepatitis, as are marked elevations of serum alkaline phosphatase activity and total white blood cell count. That the latter correlation is not due to a significant relationship between serum alkaline phosphatase activity and the degree of lymphocytosis in the peripheral blood is indicated in Figure 5.

Further consideration of the relationship between serum alkaline phosphatase activity and serum globulin concentration is facilitated by examination of cases in which multiple, concomitantly obtained values are available. Figure 6 presents such data for four cases, and illustrates the degree of parallelism that may be observed, although cases demonstrating considerably less similarity may also be encountered. In all, the present series contained eleven patients in whom three or more concomitantly obtained values for serum alkaline phosphatase activity and serum globulin concentration were available. Evaluation of these cases* indicated that determinations of these substances tended to fluctuate together to a degree which possessed a high degree of statistical significance. Although this does not necessarily signify a cause-and-effect relationship, similar concomitant fluctuations between these two substances have been pointed out in other diseases, as will be noted subsequently.

* The problem of the statistical significance of the observed concomitant fluctuations of serum globulin concentrations and serum alkaline phosphatase activity was approached by ranking the observed values by magnitude and calculating a coefficient of rank correlation [*21b*] for the observations on each patient, and an average coefficient of rank correlation for the entire group of eleven patients. The latter figure was 0.697. The variance of this quantity was calculated as 0.034, and the standard error as 0.184. Thus, the average coefficient of rank correlation for the group lay well outside ± 2 standard errors.

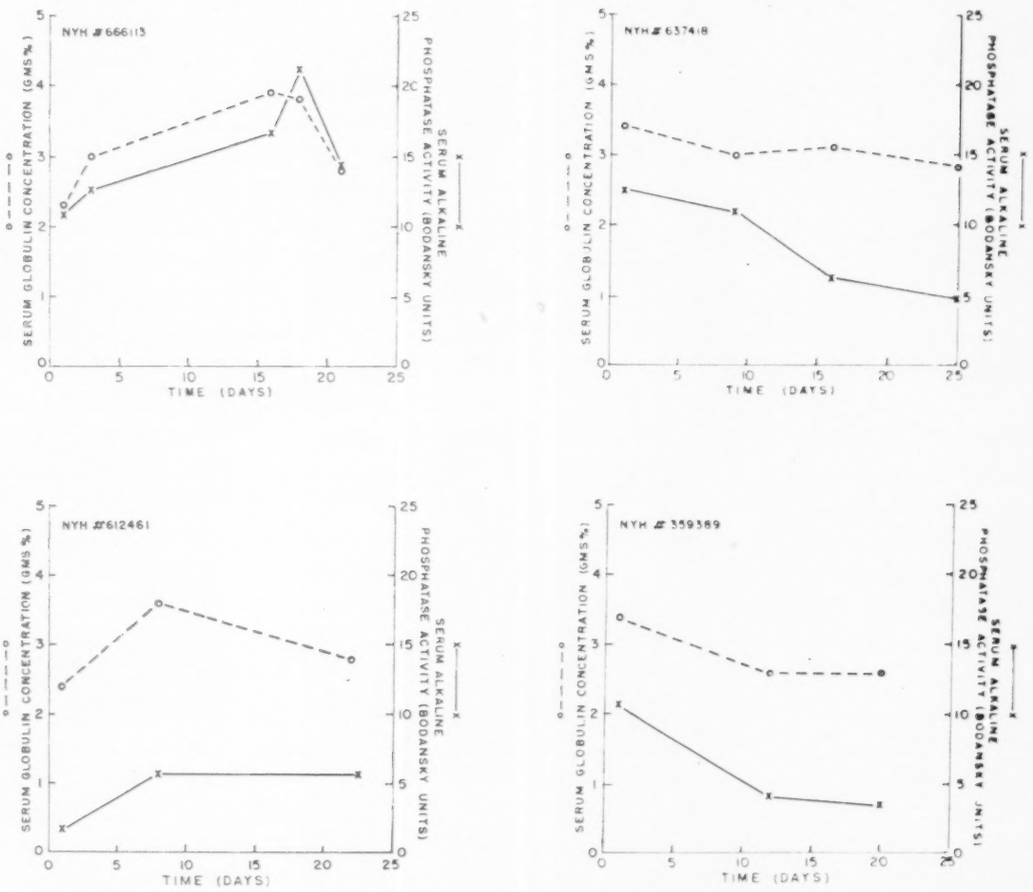


FIG. 6. Concomitant fluctuations in serum globulin concentration and serum alkaline phosphatase activity in individual cases of infectious mononucleosis.

COMMENTS

Several studies [*22–34*] have documented the high frequency with which hepatic dysfunction occurs in infectious mononucleosis: the incidence of clinical jaundice is generally reported as 2 to 12 per cent [*22,23,25,26,28,30,31,35,36*]. Marked elevations in serum alkaline phosphatase activity have been noted in cases reported by others [*24–26,31,36,37*], and, as in the cases reviewed in the present report, this finding has usually been associated with only modest elevations of serum bilirubin concentration.

The cause of such marked alkaline phosphatasemia in this disease is obscure, and this is also the case when this chemical aberration is found in a variety of other disorders, as noted by Gutman [*38*] and by Ross et al. [*17*]. In infectious mononucleosis pathological studies of the liver [*27,35,37,39*], performed chiefly on specimens obtained by needle biopsy, have demonstrated focal infiltrations within the lobules, composed primarily of mononuclear cells and associated with local hepatocellular necrosis. These infiltrates have sometimes been most marked in the portal areas, and have been associated with swelling of Kupffer cells, endothelium and the lining epithelium of small bile ducts. The latter may show diminution and sometimes complete obliteration of the caliber of their lumens. Kilham and Steigman [*22*] have reported a needle biopsy of the liver obtained on the eleventh day of the illness in a patient with a serum alkaline phosphatase activity of 26 Bodansky units and a total serum bilirubin of 1.5 mg. per cent. This specimen showed focal acute hepatitis with loss of liver cells. Changes were maximal in the portal tracts, where early proliferation of bile ducts was noted.

In Gloyne's [*37*] patient, from whom three specimens of liver were obtained over a two-month period, infiltrative foci of mononuclear cells and packing of the sinusoids with similar cells were the chief changes noted; striking abnormalities of the small bile ducts were not

found, even at a time when the serum alkaline phosphatase activity was 29 Bodansky units.

Thus marked alterations in serum alkaline phosphatase activity in infectious mononucleosis may be but are not necessarily accompanied by striking histologic evidence of alterations in small bile ducts within the liver, or by impressive lesions of the portal tracts, but rather may be associated pathologically with the presence of infiltrations of mononuclear cells elsewhere in the liver lobules. Ross, Iber and Harvey [*17*] have pointed out a similar lack of correlation between histologic evidence of lesions in the portal areas and marked increase in serum alkaline phosphatase activity in cases of sarcoidosis, Hodgkin's disease, disseminated lupus erythematosus, tuberculosis and amyloidosis; it is to be noted that each of these may cause infiltrative hepatic lesions elsewhere in the lobule.

The mechanism(s) whereby such infiltrative lesions may cause elevations in serum alkaline phosphatase activity are unclear; although this chemical finding in the serum always raises a question as to the patency of the extrahepatic or intrahepatic biliary tree with interference with excretion of alkaline phosphatase in the bile, there is a considerable body of experimental and clinical evidence suggesting that the liver may produce this enzyme. Thus, in cases such as those reported here, overproduction, rather than interference with the excretion of alkaline phosphatase, may be responsible for the high serum levels noted. Ross and his co-workers have speculated that elevation of serum alkaline phosphatase activity may be related to protein synthesis; in this regard it should be noted that each of the disorders studied by that group is frequently accompanied by elevations in serum globulin concentration, and that the present study suggests that in infectious mononucleosis a correlation exists between hyperglobulinemia and moderate or marked elevations in serum alkaline phosphatase activity.

The issue cannot be settled at this time, and Ross' summation is apt, both for his own cases and for those in the present study: "The pathogenesis of the elevation of serum alkaline phosphatase activity in this group of patients remains obscure but on the basis of the information at hand cannot be attributed to intrahepatic biliary obstruction alone" [*17*].

The possibility that obstruction of the extrahepatic biliary tree may occasionally occur in infectious mononucleosis as a result of compression of ducts by enlarged lymph nodes cannot be eliminated, since postmortem studies are uncommon in this disease. However, such obstruction of the ducts is extremely unusual in other diseases characterized by enlargement of the lymph nodes and, in addition, would be expected to cause, at least on occasion, more striking elevations in serum bilirubin and cholesterol concentrations than were observed in our cases. Moreover, the available histologic studies of the liver in infectious mononucleosis have not demonstrated changes suggesting obstruction of the extrahepatic ducts [*35*].

Finally, the possibility exists that elevation of serum alkaline phosphatase activity in infectious mononucleosis might be related to the peculiar mononuclear cells characterizing the peripheral blood and the tissue lesions in this disorder. The possibility that these cells may themselves be rich in alkaline phosphatase activity has been investigated by Wachstein [*40*] by histochemical technics; these studies have revealed no evidence of such activity in the "atypical" lymphocytes of infectious mononucleosis. Further, our own study fails to demonstrate a relationship between the height of alkaline phosphatase activity in the serum and the degree of lymphocytosis in the peripheral blood.

SUMMARY

1. Of seventy-three hospitalized patients with infectious mononucleosis in whom liver function tests were carried out, 87.7 per cent had elevations of serum alkaline phosphatase activity. These elevations were marked in 13.7 per cent of the patients.

2. In general, the illness was slightly more severe in patients with marked elevation of serum alkaline phosphatase activity.

3. The data indicate a correlation between the occurrence of hyperglobulinemia and moderate or marked elevations of serum alkaline phosphatase activity in infectious mononucleosis, as well as a correlation between the latter and the degree of leukocytosis observed.

4. The cause of elevation of serum alkaline phosphatase activity in infectious mononucleosis is not clear, but it probably does not relate exclusively to obstructive interference with hepatic excretion of the enzyme.

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The Cholestatic Form of Viral Hepatitis*

Experiences with Viral Hepatitis at Brooke Army Hospital During the Years 1951 to 1953

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As a result of the increasing use of liver biopsy during the past two decades a classic histopathologic picture for non-fatal viral hepatitis has emerged [*1–6*]. The diffuse hepatic lesion with concomitant necrosis and regeneration of liver cells, the crumbling of lobular architecture, the striking exudate in parenchyma and portal tracts, is now well known. But concurrently with the evolution of the classic entity there repeatedly cropped up the suggestion that certain patients with viral hepatitis might present the histologic appearance of cholestatic (cholangiolitic) hepatitis [*7,8*]. The latter picture, with its centrolobular bile stasis and mild parenchymal necrosis, was so different histologically from the standard case of viral hepatitis that it was difficult to believe that two such widely dissimilar lesions could be produced by the same agent. Moreover, such cases appeared only sporadically and their etiology could not be proved because there is still no conclusive laboratory test for demonstrating the virus of infectious hepatitis. The problem was further complicated by an awareness of a growing list of drugs that similarly evoked a cholestatic type of reaction [*9,10*].

We were therefore particularly struck by an unusual series of cases of hepatitis occurring at Brooke Army Hospital in San Antonio, Texas. There, among armed forces personnel and veterans, appeared a disease that was clinically indistinguishable from viral hepatitis but which presented the histologic features of cholestatic hepatitis in a large proportion of cases. This disease occurred in the form of a minor epidemic under conditions suggesting that the causative factor was some microorganism. At the same time (1951 through 1953) we studied a series of cases of serum hepatitis occurring among soldiers who had received blood products in the Far East prior to transfer to Brooke Army Hospital for their battle injuries. The patients in the latter group showed the classic histologic features of viral hepatitis. Thus we were able to study the two types of cases side by side. This paper presents our experiences with these two types of hepatitis during the years 1951 through 1953. Our studies corroborate the view that, under unusual circumstances, viral hepatitis may present the histologic picture of cholestatic hepatitis.

METHODS AND MATERIALS

Sources of Patients. During the three-year period of the study 390 patients with a wide assortment of liver diseases were examined by liver biopsy on the Gastroenterology Service of Brooke Army Hospital. The most common liver disease encountered in this group was viral hepatitis, which accounted for 116 cases. Of these 116 cases only ninety-three are included in the present report because our analysis is limited to those in which the first liver biopsy was performed within twenty-one days after onset of jaundice.

The ninety-three cases of viral hepatitis fell into two main groups. One group consisted of soldiers, civilian dependents and veterans who lived in or near Texas. Clinically, this group presented the picture of infectious hepatitis; there was no known exposure to blood products, drugs or hepatotoxins. There were forty-six patients in this group. The other group was comprised of soldiers who had received blood products in the Far East prior to being transferred to Brooke Army Hospital for treatment of their injuries. Most of the men in this group had been wounded in battle in Korea; others had suffered some accident while

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stationed in Korea or Japan. These patients were assumed to have had serum hepatitis because the long incubation period was compatible with this immunologic variety. There were thirty patients in this group.

There were also eight patients who presumably contracted infectious hepatitis in the Far East and in whom symptoms developed after arriving in southwestern U.S.A. Another group was comprised of six residents of Texas in whom serum hepatitis developed after receiving blood products.

For three patients there was no record of the geographic residence during the incubation period of the disease.

Management of Patients. All patients were treated by Col. B. H. Sullivan and his staff on the Gastroenterology Service, Brooke Army Hospital, in the following manner. Patients were kept at bedrest with bathroom privileges and were given a high protein, high calorie diet. Ambulation was allowed when the total serum bilirubin returned to 1 mg. per cent and the bromsulfalein retention dropped to 5 per cent or less forty-five minutes after a 5 mg. per kilo dose of dye. If the results of these tests remained normal for two weeks after ambulation was begun, the patient was either allowed to go on convalescent leave, transferred to another service or, if a civilian, discharged from the hospital. A few patients received ACTH intravenously (20 units daily for ten days, then a tapering dose for seven days). The following laboratory tests were performed serially on all patients: total leukocyte count, direct reacting and total serum bilirubin, thymol turbidity, serum alkaline phosphatase and bromsulfalein retention. Needle biopsy of the liver was performed in each case in the acute phase of the disease; in most cases this was followed by one or more liver biopsies later in the course of the disease.

Histologic Analysis. In order to determine whether or not there were any correlations between the histologic features and other aspects of the disease, the cases were divided into histologic types on the basis of the histopathologic changes. The following features were recorded for each biopsy specimen:

(1) The time interval between onset of jaundice and biopsy.

(2) The appearance of the intrahepatic bile ducts, both interlobular ducts and larger collecting ducts. Also, estimates were made of the degree of ductular proliferation, using an arbitrary grading of 1 to 4 plus.

(3) The severity of inflammation of the portal areas. This was graded arbitrarily from 1 to 4 plus. Special attention was paid to the relative proportion of mononuclear cells, eosinophils and polymorphonuclear leukocytes.

(4) The severity and distribution of the parenchymal lesion. The degree of necrosis and inflammation was graded from 1 to 4 plus. It was also noted whether the lesion was diffuse or focal in the specimen. The types of inflammatory cells in the lobules were recorded and an attempt was made to determine the relative proportion of these cells.

(5) The degree of intrahepatic bile stasis. We recorded the presence of bile-staining of liver cells as well as the size, shape, number and zonal localization of bile plugs. The degree of intrahepatic bile stasis was graded from 1 to 4 plus. Mere bile-staining of liver cells (without intracanalicular bile plugs) was graded as "zero," and rare plugs as "plus-minus." "One plus" consisted of a few bile plugs (3 to 10) in about half the liver lobules; "2 plus" designated 10 to 20 bile plugs in all liver lobules; "3 plus" designated more than 20 plugs in the centrolobular zones of the lobules. There were no cases of "4 plus" bile stasis, which grading was reserved for the severe degree of bile stasis that occurs in extrahepatic biliary obstruction of about four to six weeks' duration.

The presence of lipofuscin in macrophages was demonstrated with Mallory's basic fuchsin stain and the use of periodic acid-Schiff reagent with digestion. Gomori's stain for hemosiderin with kernechtrot counterstain demonstrated any hemosiderin in macrophages and also served to highlight the presence of any bile plugs.

Classification of Histologic Types. By using the histologic criteria mentioned, we divided the cases into four histologic types. This same classification had been applied to a group of control cases of viral hepatitis from other military installations; these have been described elsewhere [*11*]. In the present study the classification was restricted to those cases in which the first biopsy was performed within twenty-one days after the onset of clinical jaundice, because it is in this acute phase of the disease that the histologic features are full blown and most distinctive. Later in the disease the distinctions become blurred. Ninety-three cases were so classified into the following four histologic types.

Type A. Diffuse necrosis and inflammation of parenchyma, with absent or minimal intrahepatic bile stasis (classic viral hepatitis).

Type B. Diffuse necrosis and inflammation of parenchyma but with superimposed bile plugs (classic viral hepatitis with intrahepatic cholestasis). (Figs. 1 and 2.)

Type C. Cholestatic hepatitis, with slight to moderate focal necrosis and inflammation of parenchyma. In this type there is a moderate degree of intrahepatic bile stasis. In contrast to the diffuse and generally severe parenchymal lesion in types A and B, the lobular necrosis and inflammation in type C is only focal. (Figs. 3 to 8.)

Type D. Cholestatic hepatitis, with negligible parenchymal necrosis and inflammation. In this type the lesion is comprised almost exclusively of bile stasis and pericholangitis. (Figs. 9 and 10.)

These four histologic types represented a spectrum of histologic reactivity, so that assignment of any given case to one of these four types was not always

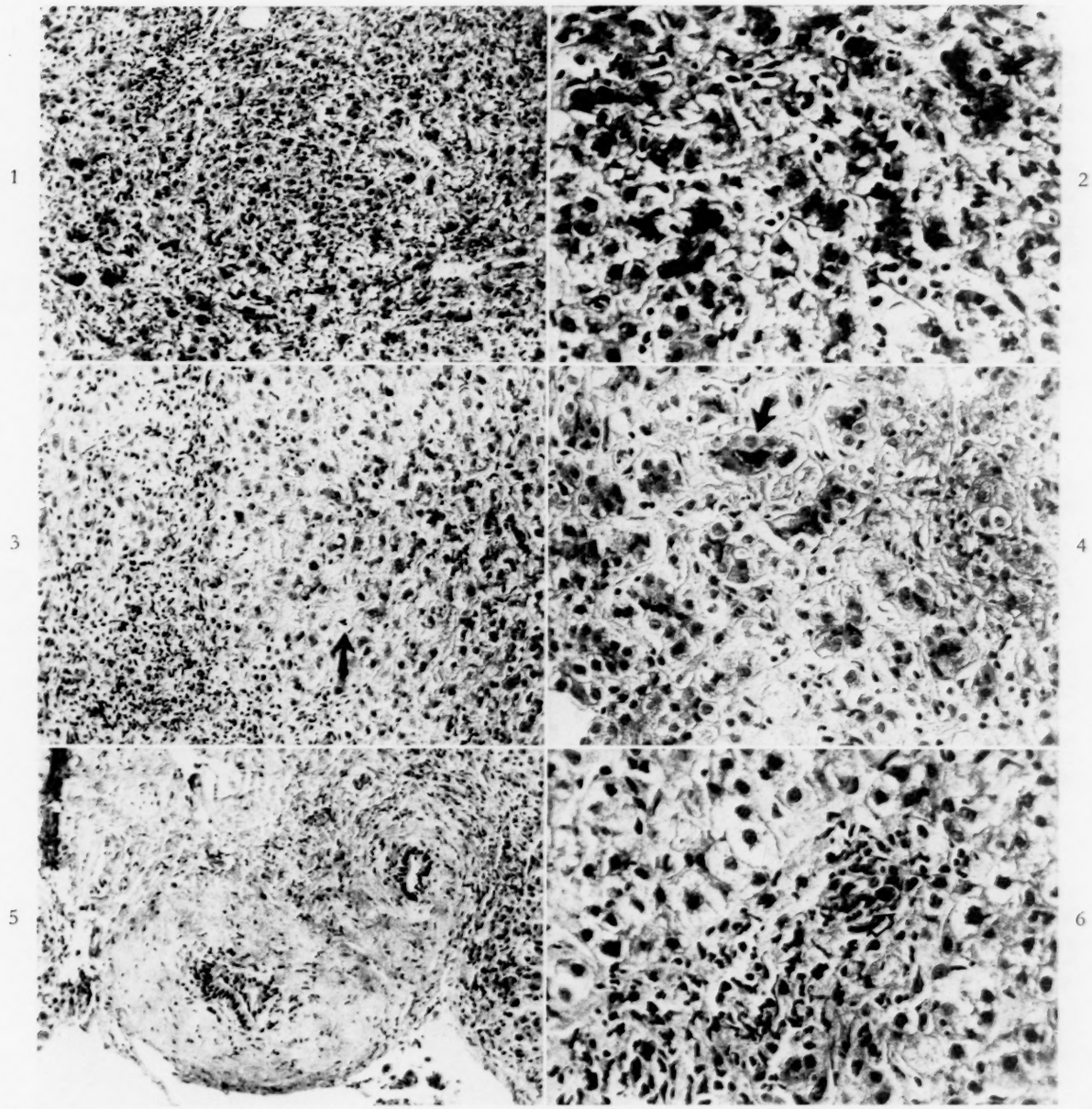


FIG. 1. Histologic type B. Biopsy was performed three days after onset of jaundice in a twenty-one year old man with serum hepatitis. The appearance is that of the classic variety of viral hepatitis with intrahepatic bile stasis. Hematoxylin and eosin × 130. Histologic type A was identical except that intrahepatic cholestasis was lacking.

FIG. 2. A centrolobular zone from same specimen shown in Figure 1. The *solid arrows* point to four large intracanalicular bile plugs. Many damaged liver cells show bile-staining of the cytoplasm (*outlined arrow*). Hematoxylin and eosin × 440.

FIG. 3. Histologic type C; cholestatic type of viral hepatitis with focal necrosis of parenchyma. Biopsy was performed three days after onset of jaundice in a twenty-five year old man with infectious hepatitis. There is mild focal necrosis and inflammation of parenchyma; the portal area contains many inflammatory cells. The *arrow* points to one of many slender bile plugs (approximately 30) located centrolobularly. Hematoxylin and eosin × 180.

FIG. 4. Histologic type C; cholestatic hepatitis. The *arrow* points to one of many bile plugs of various sizes and shapes that lie in a centrolobular zone. Biopsy was performed thirteen days after onset of jaundice in a twenty-one year old man with serum hepatitis. Hematoxylin and eosin × 350.

FIG. 5. A large collecting intrahepatic bile duct that was contained in the same biopsy specimen portrayed in Figure 4. This duct is histologically normal. Compare it to the interlobular duct shown in Figure 6. Hematoxylin and eosin × 165.

FIG. 6. From same biopsy specimen shown in Figures 4 and 5. The interlobular duct (*arrow*) of the portal area is surrounded by polymorphonuclear leukocytes; its lumen, however, is free of inflammatory cells. Hematoxylin and eosin × 440.

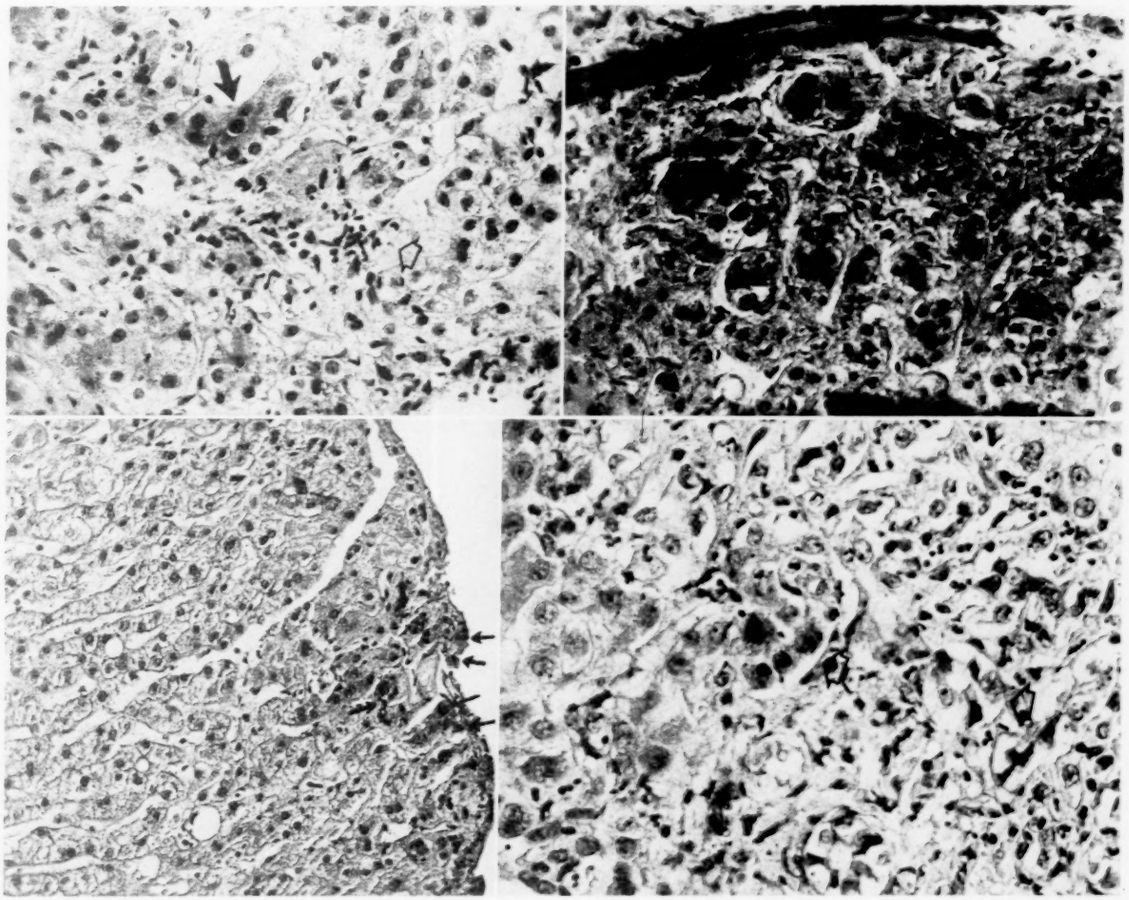


FIG. 7. Histologic type C; cholestatic hepatitis. There is an area of focal necrosis of parenchyma containing mononuclear cells and polymorphonuclear leukocytes (*outlined arrow*). The *solid arrow* points to a round bile plug surrounded by a tubular formation of liver cells. Original magnification × 395.

FIG. 8. Severe intrahepatic bile stasis, grade 3, in a case of cholestatic hepatitis, histologic type C. Biopsy was performed seven days after onset of jaundice in a young woman with infectious hepatitis. The bile plugs (which have been outlined in ink) are large and numerous. The over-all histologic appearance resembles that seen in some cases of extrahepatic biliary obstruction. This patient recovered in about three and a half months. Hematoxylin and eosin × 395.

FIG. 9. Histologic type D; mild cholestatic hepatitis with slight intrahepatic cholestasis and negligible parenchymal necrosis. Biopsy was performed four days after onset of jaundice in a forty-two year old man with infectious hepatitis. The *arrows* point to several tiny bile plugs in the centrolobular zone. Hematoxylin and eosin × 210.

FIG. 10. Portal area from same biopsy specimen shown in Figure 9. There is slight ductular proliferation. The ductules show swelling of epithelial cells and are surrounded by small numbers of polmorphonuclear leukocytes (*arrows*). Hematoxylin and eosin × 440.

accomplished with strict accuracy. The greatest difficulty was encountered in separating types B and C; the decision rested mainly on the degree and extent of necrosis.

Microbiologic Studies. A search was made for pathogenic microorganisms in fourteen cases selected at random. Blood cultures were obtained. When a needle biopsy was performed a portion of the specimen was processed for histologic study and the remainder placed in nutrient broth. Cultures for bacteria were set up on standard bacteriologic media, and the specimen was then stored frozen in the dry ice chest for later virus studies. Prior to animal inoculation the liver specimen was emulsified with mortar and pestle and made into a 20 per cent suspension with sterile inactivated 10 per cent rabbit serum, without centrifugation. Litters of suckling mice (twenty-four to seventy-two hours old) were inoculated intraperitoneally with 0.05 ml. of the suspension. Six to ten days later, liver tissue from the surviving mice was harvested; some of it was used for histopathologic study and some emulsified as before in rabbit serum and passed to other litters of suckling mice. The mice of the fourth passage were observed for thirty days,

and were then killed; part of each liver was studied histologically and the remainder stored in the deep freeze chest for future reference.

RESULTS AND INTERPRETATION

Histopathology

The ninety-three cases were distributed as follows among the four histologic types: type A, twenty-eight cases; type B, thirty-nine cases; type C, twenty-two cases; type D, four cases.

In the cases comprising type A the patients showed the classic picture of viral hepatitis, namely, diffuse inflammation and piecemeal necrosis of parenchyma, concomitant degeneration and regeneration of liver cells, lobular disintegration, scattered pigmented macrophages, and infiltration of portal tracts with inflammatory cells. (Fig. 1.) Intrahepatic bile stasis was absent or negligible.

The inflammation and piecemeal necrosis of the parenchyma involved the lobules in a fairly diffuse manner. Using our arbitrary grading system, the degree of necrosis and inflammation had a mean value of 2 to 3 plus. Most of the cells infiltrating the lobules were of the mononuclear type, and only few eosinophils were present. Portal inflammation was invariably present, and was of 3-plus severity. The cells in the portal tracts were mainly mononuclear cells; some eosinophils were present in almost all cases, but polymorphonuclear leukocytes were sparse.

Pigmented macrophages were diffusely scattered through the lobules. Some of these phagocytes were clearly Kupffer cells while others were more rounded and may have been altered Kupffer cells or phagocytes from other sources. The pigment was of two types, hemosiderin and lipofuscin. Normally the liver cells contain ferritin and lipofuscin. Presumably when liver cells die they release these substances which are then engulfed by the Kupffer cells. The ferritin is apparently changed to hemosiderin by the Kupffer cells; the lipofuscin may also be somewhat altered in like manner. As time passes, these pigmented macrophages gradually leave the lobule, and some are later found in the portal tracts. This suggests that the macrophages move towards the periphery of the lobules, perhaps by way of the space of Disse which may serve as a sort of intralobular lymphatic.

"Ductular proliferation" was seen at the periphery of liver lobules; the degree varied from 0 to 3 plus, with a mean value of 1 plus for type A. We had the impression that most of these new ductules consisted of liver cells differentiating into tubular structures rather than representing branchings of preexisting interlobular ducts, but we made no special study of their origin. The interlobular bile ducts of the portal areas were surrounded by inflammatory cells and occasionally showed swelling of the epithelium. This may signify that the interlobular ducts were damaged. On the other hand, "ductular proliferation" so often reflects injury to liver cells, and the ubiquitous presence of inflammatory cells in portal tracts may simply represent a drainage of these cells from the lobules or an injury to other structures of the portal tracts, such as collagen bed or blood vessels or lymphatics. Consequently, we are inclined to believe that there was no significant damage to the interlobular bile ducts. Also, in five cases the biopsy specimen contained medium sized collecting bile ducts, and these were normal.

The histologic findings in type B were similar to those in type A, with the following exceptions. (Fig. 1.) (1) Intrahepatic bile stasis was present in the centrolobular zones (Fig. 2); in half the patients bile stasis was 1-plus and in the other half 2-plus. In most cases the bile plugs were narrow and elongated, but in about a fourth of the cases the bile plugs were more rounded and somewhat larger. (2) There were more polymorphonuclear leukocytes in the portal tracts. Although mononuclear cells still predominated, the cellular exudate contained about 30 per cent polymorphonuclear leukocytes in about one-fourth of the patients. (3) A few polymorphonuclear leukocytes were present in the exudate within the lobules.

In type C, parenchymal necrosis and inflammation were less severe and more focal than in types A and B. The degree of necrosis was approximately half of that in types A and B. (Compare Figure 1 with Figs. 3 and 7.) The intralobular exudate was still comprised chiefly of mononuclear cells; polymorphonuclear leukocytes were sparse. The exudate in the portal areas contained proportionately more polymorphonuclear leukocytes than in types A and B; although mononuclears were still prominent and eosinophils were clearly present, polymorphonuclear leukocytes comprised about half of the cellular exudate in approximately half of the cases. (Fig. 6.)

There were fewer phagocytes containing

TABLE 1
INCIDENCE OF INTRAHEPATIC BILE STASIS IN CLASSIC AND CHOLESTATIC TYPES OF VIRAL HEPATITIS, IN RELATION TO TIME INTERVAL AFTER ONSET OF CLINICAL JAUNDICE

Time Interval after Onset of Jaundice	Classic Hepatitis (types A plus B combined)		Cholestatic Hepatitis (types C plus D combined)	
	Total No. of Biopsy Specimens	No. with Bile Stasis	Total No. of Biopsy Specimens	No. with Bile Stasis
0–4 days	13	6	3	3
5–7 days	17	9	8	8
8–10 days	15	8	5	5
11–14 days	8	6	5	5
15–21 days	12	7	4	4
22–28 days	4	3	0	0
29–35 days	5	1	0	0
5–8 weeks	9	1	5	2
2–6 months	24	0	6	0
6 months or more	5	0	1	0

hemosiderin and lipofuscin, presumably because fewer liver cells had undergone necrosis.

The degree of ductular proliferation was about half of that in types A and B. Perhaps this is also a reflection of less intense liver cell necrosis, so that there was less stimulus for this type of tubular regeneration on the part of liver cells. The interlobular bile ducts occasionally showed swelling of the lining epithelial cells, but as in the classic histologic variety (our types A and B), we saw no obvious lesions of these structures. (Figs. 6 and 10.) In two cases the biopsy specimens contained medium sized bile ducts, and in two others, larger collecting bile ducts. In all four cases these ducts were normal (Fig. 5), suggesting as before that there probably was no disease of the intrahepatic biliary system.

The degree of intrahepatic bile stasis was more pronounced in type C as compared to type B. (Figs. 3, 4 and 8.) In eight cases intrahepatic cholestasis was graded as 1 plus, in eleven cases as 2 plus, and in four cases as 3 plus. On the average the bile plugs were somewhat larger than those seen in type B. In half the cases the plugs were thin and elongated, but in the other half the plugs were rounder and larger. In the four cases in which bile stasis was 3 plus there was one in which the bile plugs were large and round, similar to that seen in some cases of obstructive jaundice (Fig. 8); in this case, on histologic grounds alone, obstruction of the extrahepatic biliary tract could not be excluded. Where bile canaliculi were distended by such larger plugs, the surrounding liver cells took on a tubular appearance. (Fig. 7.)

Type D was exemplified by only four cases. Altogether, the histologic abnormalities were less severe than in type C. (Figs. 9 and 10.) Parenchymal necrosis and inflammation were minimal. The intralobular cellular exudate was scanty; mononuclear cells predominated, although some polymorphonuclear leukocytes were present. In the portal areas, however, the polymorphonuclear leukocyte was the predominant cell. The amount of cellular exudation in the portal regions was about half that present in histologic type C. Ductular proliferation was mild. Intrahepatic cholestasis was also of mild degree: it was rated as 1 plus in three cases and 2 plus in one case; all bile plugs were small and slender.

Incidence of Intrahepatic Cholestasis with Respect to Phase of Disease. One of us (I. N. D.) has already reported a study on intrahepatic cholestasis in a group of patients with classic viral hepatitis, which served as a control group for the present series of cases. In the control group about 40 per cent of patients showed intrahepatic cholestasis in the acute phase of the disease, the peak occurring five to fifteen days after onset of jaundice [*11*].

In order to have an adequate basis for comparing the present series of cases with the control group, we combined types A and B into one category and types C and D into another. Histologically, the cases in types A and B of the present series were identical with those of the control group, while the cases in types C and D formed an entirely different category.

The results are shown in Table 1. In the category comprised of type A plus B, intrahepatic bile stasis occurred in about 50 per cent of cases during the first ten days after clinical onset of jaundice. The incidence appeared to be somewhat higher (from 60 to 75 per cent) in the specimens obtained eleven to twenty-eight days after onset of jaundice. After four weeks the incidence of intrahepatic cholestasis fell off sharply, reaching zero in specimens obtained after eight weeks. Thus, in the present series of cases of classic viral hepatitis (types A plus B)

TABLE II
AGE, SEX AND RACE OF PATIENTS IN THE FOUR HISTOLOGIC TYPES

Histologic Type	Average Age (yr.)	Sex		Race			Total
		Men (no.)	Women (no.)	White Patients (no.)	Negro Patients (no.)	Unknown (no.)	
A	23.4	26	2	26	2	0	28
B	25.8	39	0	34	3	2	39
C	28.0	20	2	22	0	0	22
D	27.0	4	0	3	1	0	4
Total	25.6	89	4	85	6	2	93

the incidence of intrahepatic cholestasis appeared to be higher during the first month after onset of jaundice, as compared with that of the control group [*11*]. Because of the limited number of cases in the present series, one cannot be certain that this difference is statistically significant. In both series of cases of classic hepatitis, intrahepatic cholestasis tapered off rapidly after the first month of jaundice and virtually disappeared by eight weeks.

In the cases comprising the category of cholestatic hepatitis (type C plus type D) intrahepatic cholestasis persisted in all cases in which biopsy was performed up to twenty-one days after jaundice began. Unfortunately no follow-up biopsy specimens were obtained in the period twenty-two to thirty-five days after onset of jaundice. In the time interval five to eight weeks after the clinical onset of jaundice five follow-up biopsy specimens were obtained and two of these showed intrahepatic cholestasis. No bile stasis was seen after eight weeks.

Correlation Between Histopathologic and Clinical Features

After the cases had been divided into the four histologic types, we analyzed them to determine whether or not there were any correlations between histologic grouping and clinical features.

Age, Sex and Race. Table II shows the age, sex and race distribution among the four histologic types. Since our patients were mainly young soldiers, it was expected that the average age was approximately the same for the various histologic types. Because our series contained only four women and six Negroes, we were unable to learn whether sex or race played any role in the histologic reactivity.

Epidemiology. An analysis was made of any possible correlation between histologic type and (1) geographic residence of the patient during the incubation of the disease, and (2) "immunologic" type of viral hepatitis, that is, whether the patient had infectious hepatitis or serum hepatitis. For the purposes of this study we assumed that a patient had the serum hepatitis variety of the disease if he had received blood products two to six months prior to onset of his disease. The results are shown in Table III.

TABLE III
NUMBER OF PATIENTS IN THE FOUR HISTOLOGIC TYPES, GROUPED ACCORDING TO THE IMMUNOLOGIC TYPE OF VIRAL HEPATITIS AND THE GEOGRAPHIC AREA WHERE INFECTION WAS CONTRACTED

Histologic Type	Far East (Korea and Japan)		Texas, U.S.A.		Total
	Infectious Hepatitis	Serum Hepatitis	Infectious Hepatitis	Serum Hepatitis	
A	5	11	8	1	25*
B	3	14	17	5	39
C	0	4	18	0	22
D	0	1	3	0	4
Total	8	30	46	6	90

* There were three patients with infectious hepatitis showing histologic type A lesion for whom the geographic residence was uncertain.

For purposes of statistical analysis we placed types A and B into one category, designated "classic viral hepatitis," and types C and D into another category, "cholestatic hepatitis." First we wanted to determine whether there was any relationship between the type of infection (serum hepatitis or infectious hepatitis) and the histologic groupings. Application of the chi-square test and the t test indicated that there was some kind of relationship between the type of infection and the histologic picture.* Thus, of twenty-six cases comprising the category of cholestatic hepatitis (type C plus D) twenty-one (80.8 per cent) were of the infectious hepatitis type and five of the serum hepatitis variety. Of the sixty-four patients comprising the category of classic viral hepatitis (type A plus B), thirty-three (51.6 per cent) had infectious hepatitis and

* The chi-square value was 3.81, which falls just at the 0.05 level and is therefore probably significant. The value of t was 2.19, with a probability of 0.03.

TABLE IV

RESULTS OF ANALYSIS VARIANCE TEST APPLIED TO VARIOUS CLINICAL FACTORS COMPARED IN THE FOUR HISTOLOGIC TYPES

Factor	Mean of Group				Value of F	Statistical Significance
	Histologic Type A	Histologic Type B	Histologic Type C	Histologic Type D		
No. of weeks between onset of jaundice and biopsy	2.25	1.87	2.38	1.50	1.57	Not significant
White blood cells* (thousand cells per cu. mm. of blood)	7.84	7.08	7.24	7.17	0.12	Not significant
Thymol turbidity units*	15.11	9.94	14.95	10.73	1.51	Not significant
Serum alkaline phosphatase* (Bodansky units)	8.62	9.74	6.89	19.87	5.87	Significant at .01
Direct serum bilirubin* (mg./100 ml.)	3.40	7.16	5.11	1.20	2.91	Significant at .05
Total serum bilirubin* (mg./100 ml.)	5.50	10.56	9.45	3.47	3.53	Significant at .05
No. of days in hospital	61.91	77.54	80.18	†	1.65	Not significant
No. of weeks for return of bromsulfalein test to normal	7.70	9.70	5.56	4.00	2.72	Not significant

* Admission values.

† This factor could not be analyzed. In two cases the hospitalization days were thirty and thirty-four, respectively; a third patient left without permission after thirteen days, but he appeared to be only mildly ill from the beginning and recovering quickly; in the fourth case we have no accurate record of the length of hospitalization.

thirty-one (48.4 per cent) serum hepatitis. At first glance this would seem to suggest that infectious hepatitis is more apt to cause the cholestatic type of hepatitis and that serum hepatitis is more likely to produce the classic variety of viral hepatitis. We know that this is generally not true (as will be shown later), so we had to look for another explanation.

The answer lay in the place of residence where the patients contracted their infection. Thus there were thirty cases of serum hepatitis from the Far East; of these, twenty-five patients (83.3 per cent) presented the histologic picture of classic hepatitis and five (16.7 per cent) the picture of cholestatic hepatitis. Eight patients contracted infectious hepatitis in Korea and were then transferred to Brooke Army Hospital; all of these showed the histologic picture of classic hepatitis. There were six patients residing in Texas in whom serum hepatitis developed; all of these showed the histologic picture of classic hepatitis. There remain forty-six patients who contracted infectious hepatitis in Texas. Of these, twenty-five (54.3 per cent) showed the histologic features of classic hepatitis and twenty-one (45.7 per cent) the features of cholestatic hepatitis. A statistical analysis indicated that there was a significant relationship between geographic residence and immunologic type of hepatitis (infectious hepatitis or serum hepatitis). In applying the chi-square test, the probability was less than 0.001, indicating that the differences were statistically significant.

Thus of the twenty-six cases of cholestatic hepatitis twenty-one were cases of infectious hepatitis contracted in Texas. The other five were presumably examples of serum hepatitis contracted in the Far East, although it is possible that in some or all of these the patients may have contracted infectious hepatitis in Texas after their transfer from the Far East. These results indicated, therefore, that the patients residing in Texas contracted a form of infectious hepatitis that had a propensity for evoking a cholestatic type of hepatitis.

Laboratory Tests. A statistical analysis was made of the values of the following tests that had been performed when the patients were first admitted to the hospital: total leukocyte count, thymol turbidity, serum alkaline phosphatase and serum bilirubin (direct and total). (Table IV.) There was no significant difference in the mean values of either the total leukocyte count or the thymol turbidity test between the four histologic types. As regards the serum alkaline phosphatase values, there was no significant

difference between types A, B and C; the mean value for type D was twice that of the others and this difference was significant, but since there were only four cases in this group we cannot be certain that this difference was meaningful.

The mean values of serum bilirubin (both the direct and total) showed significant differences between the types. In types B and C, in which intrahepatic cholestasis was present, the mean values of both the direct and total serum bilirubin were twice that found in type A, in which there was no intrahepatic cholestasis. This finding indicates that there was a correlation between the level of serum bilirubin and the presence of intrahepatic cholestasis. Patients with type D histologic picture (intrahepatic cholestasis with negligible parenchymal necrosis) had the lowest levels of serum bilirubin. Clinically all four patients comprising this group had a milder disease symptomatically; also, the degree of intrahepatic cholestasis was less than that in those with types B and C. Perhaps this again indicates a correlation between the mildness of the illness, the relative absence of liver cell necrosis, the slight degree of intrahepatic cholestasis and the mild hyperbilirubinemia. In this connection it is noteworthy that in two cases of anicteric hepatitis occurring in our series the patients showed no intrahepatic cholestasis, a phenomenon reported by Mallory in 1947 [*4*]. The correlation of intrahepatic cholestasis with the level of serum bilirubin in viral hepatitis has been discussed more fully elsewhere [*11*].

Clinical Course. Clinical appearance: There appeared to be no difference in the severity of signs and symptoms between types A, B and C. The four patients with type D histologic picture, however, did not appear to be as sick as those with the other types.

Rate of recovery: For the purposes of this study, recovery was assumed to have occurred when (1) the total serum bilirubin returned to 1 mg. per cent, and (2) when the bromsulfalein retention fell to 5 per cent. Using these criteria for the endpoint of the illness we found no significant difference in the mean values of the duration of illness among types A, B and C. These mean values are indicated in Table IV by the duration of hospitalization and the length of time it took for the bromsulfalein retention to return to normal. The four patients with type D histologic picture appeared to be less sick and to recover faster, but the data are inadequate to permit a categorical statement.

All patients recovered completely; there appeared to be no harmful sequelae. A follow-up questionnaire was sent to all patients in 1953 and 1954. The majority of patients replied, and those that did all claimed to be in good health.

Follow-up tissue studies: Fifteen patients with type A histologic picture were studied by needle biopsy performed one to five months after onset of jaundice. In none did we find any significant histologic abnormalities in the later specimens. In some patients there was mild focal necrosis of parenchyma or slight portal scarring. One patient with anicteric hepatitis had a more prolonged illness as evidenced by a bromsulfalein retention of 14 per cent at three and one half months and by focal necrosis of liver in the biopsy specimen at five months.

Nineteen patients with type B histologic picture were studied by needle biopsy two to fifteen months later. The histologic changes were mild, as in type A. Three patients showed a more prolonged disease as judged by liver function tests and the presence of focal necrosis of liver. All three, however, recovered from their hepatitis within six months; one died of myocardial infarction fourteen months after the onset of the viral hepatitis, and at autopsy his liver appeared normal.

Follow-up biopsy specimens were obtained from nine patients with type C histologic picture. Again the histologic changes were minimal. Two patients showed a more prolonged course but recovered fully within six months. In two other patients we had the opportunity to study the liver two and three years, respectively, after recovery from hepatitis. In both of these the illness had been of average severity and duration. One patient had a colectomy three years after the hepatitis; at operation his liver was grossly normal. The other died two years after the hepatitis as a result of subarachnoid hemorrhage caused by hypertension; at autopsy the liver was normal except for slight fatty metamorphosis.

No follow-up biopsy specimens were obtained from patients with type D histologic picture.

ETIOLOGY

The question arose as to the etiology of the hepatitis occurring in the Texas residents who had not received blood products, particularly since approximately half of these showed the histologic picture of cholestatic hepatitis. This high incidence of the latter type of reaction was unprecedented in published reports on viral

TABLE V

INCIDENCE OF IMMUNOLOGIC TYPES OF VIRAL HEPATITIS IN VARIOUS HOSPITALS CONTRIBUTING CASE REPORTS TO THE REGISTRY OF HEPATIC PATHOLOGY (THESE FIGURES REPRESENT ALL BIOPSY CASES THAT HAD BEEN ACCESSIONED BY THE REGISTRY AS OF OCTOBER 1957)

Hospital Sources	Total No. of Patients	Infectious Hepatitis		Serum Hepatitis		Type of Hepatitis not Stated (no.)
		No. of Patients	Per cent	No. of Patients	Per cent	
European Command	330	321	97.3	8	2.4	1
Far East Command	236	228	96.6	7	3.0	1
Walter Reed Army Medical Center	84	32	38.1	52	61.9	0
All others*	511	397	77.7	112	21.9	2
Total	1,161	978	84.3	179	15.4	4

* These sources include other military hospitals, veterans hospitals and civilian hospitals.

hepatitis. Indeed, our experience remained unique until Smetana later observed a similarly high incidence in the Delhi epidemic of 1955 and 1956 [*12*].

The following explanations were considered.

Microbiologic Agents. We considered the possibility that this unusual form of hepatitis was caused by bacteria or by viruses unrelated to the usual viruses causing hepatitis (infectious hepatitis or serum hepatitis). The results of our microbiologic studies were not helpful in this regard. By chance, of the fourteen cases so studied thirteen turned out to be of the classic histologic type and only one of the cholestatic type. Nevertheless, blood cultures were negative in all fourteen cases and in all of these isolation studies on fresh liver tissue were negative for bacteria and for viruses pathogenic for suckling mice. These studies left the question of etiology unanswered.

Influence of Timing of Biopsy. The question arose as to whether the histologic reaction of viral hepatitis could change with time so that this disease might present the classic appearance in one phase and the cholestatic form in another. If this transformation in histologic pattern could occur, the time at which the biopsy was performed would influence the classification of our cases into the various histologic types.

All evidence points to the conclusion that such a change over in histologic reactivity does not occur. An examination of the time intervals between the onset of jaundice and biopsy indicates that there was no significant difference between the mean values of the four histologic types. (Table IV.) The mean values of this time interval for types A, B, C and D were 15.8, 13.1, 16.7 and 10.5 days, respectively. That is, the first biopsy specimen on which the histologic classification was based was obtained in the acute phase of the disease in all cases.

Repeat biopsy specimens in individual cases during the acute phase of the disease were obtained from only five patients. Three of these had type A histologic picture and two type B. In all five the basic histologic pattern remained unchanged during the acute phase.

Much more significant is the experience of one of us (I. N. D.) in the Registry of Hepatic Pathology with hundreds of patients with viral hepatitis examined by serial biopsies. In no instance was a patient observed who showed a transformation of the histologic pattern during the acute phase from the classic type to the cholestatic type, or the reverse. The amount of intrahepatic cholestasis may vary as between the first and second fortnight of the disease, but the basic histologic type remains unaltered, once initiated.

Is There a Difference Between the Histologic Pictures of Infectious Hepatitis and Serum Hepatitis? Since the group of patients with serum hepatitis contracted in the Far East had a much lower incidence of the cholestatic pattern than the group with infectious hepatitis contracted in Texas, we might explain our results by assuming that, in general, serum hepatitis is more likely to produce the histologic pattern of classic hepatitis, and infectious hepatitis is more apt to evoke the cholestatic reaction. This view,

however, would be completely contrary to the experience of others and to our own extensive experience in the Registry of Hepatic Pathology. Most authors state that there is no difference in the histologic patterns of infectious hepatitis and serum hepatitis [*6,13*]. Our own experience tends to bear this out, with some reservations. A survey of cases of viral hepatitis in the Registry of Hepatic Pathology indicates that in both infectious and serum hepatitis the majority of patients (at least 90 per cent) showed the classic histologic pattern. For example, in the study on the control group that has already been reported [*11*], we selected eighty-six cases at random from Walter Reed Army Medical Center and from our military installations in Europe and the Far East. Seventeen patients with infectious hepatitis from the Far East and twenty-five from Europe all showed the classic histologic pattern. Of forty-four patients from Walter Reed Army Medical Center (of whom thirty had serum hepatitis and fourteen infectious hepatitis), forty-three showed the classic histologic type and only one had the cholestatic variety.

Table v indicates that cases of hepatitis occurring in our personnel stationed in Europe and the Far East were predominantly of the infectious variety (97.3 and 96.6 per cent, respectively). However, such patients showed a low incidence of the cholestatic type, namely 3 and 2 per cent, respectively. (Table vi.) Even in the group of patients from Walter Reed Army Medical Center (Table v), which contained a high proportion of those with serum hepatitis (61.9 per cent),* the incidence of the classic histologic type remained paramount, about 92 per cent. (Table vi.)

Thus, as a general rule, in both infectious and serum hepatitis, the overwhelming majority of patients present the classic histologic pattern. Therefore, our results at Brooke Army Hospital cannot be explained by the assumption that the usually prevalent strains of infectious hepatitis and serum hepatitis virsuses differ greatly in their ability to evoke a cholestatic type of reaction.

Drugs and Toxins. In the present series we included no case in which a patient had been exposed to a known hepatotoxin or had received a drug known to cause liver damage. Thus we excluded one patient who had received carbarsone and another who had been given propylthiouracil.

TABLE VI

INCIDENCE OF CHOLESTATIC TYPE OF VIRAL HEPATITIS IN PATIENTS STUDIED BY BIOPSY DURING THE YEARS 1951 TO 1953 INCLUSIVE AND SUBMITTED TO THE REGISTRY OF HEPATIC PATHOLOGY, GROUPED ACCORDING TO MILITARY INSTALLATION

Military Installation	Total No. of Patients with Viral Hepatitis	No. of Patients Showing Cholestatic Type	Per cent Patients Showing Cholestatic Type
European Command	121	4	3.3
Far East Command	189	4	2.1
Walter Reed Army Medical Center	77	6	7.8
Brooke Army Hospital (Far East cases)	38	5	13.2
Brooke Army Hospital (Texas cases)	52	21	40.4
Total	477	40	

NOTE: In this table no separation was made between infectious and serum hepatitis.

Altered Constitution of the Hosts. Since various factors (such as age, sex and pregnancy) may affect the reaction of the host in viral hepatitis [*14–16*], we considered the possibility that some peculiar environmental factor might have altered the reactivity of the hosts. But there was nothing in our findings to suggest that this may have occurred. Almost all our Texas residents who came down with infectious hepatitis were either young soldiers on standard army diet or young veterans living near San Antonio, Texas. Presumably, these men were previously healthy, well nourished young adults who had not been subjected to any particular environmental factor that might have influenced their reactivity to the agent that caused their hepatitis.

Conclusions as to Etiology. After considering these various possibilities we were led to the conclusion that the etiologic agent was probably a virus that was related to the virus of infectious hepatitis. Of course we cannot exclude the possibility that the etiologic agent was immunologically distinct from both infectious hepatitis and serum hepatitis viruses. But since these cases were remarkably similar to those of viral hepatitis we prefer the view that the etiologic agent was a variant of infectious hepatitis virus. It seems likely that there may be substrains of both infectious hepatitis virus and

* As in the case of Brooke Army Hospital, many of these patients were soldiers who had been wounded in Korea, received blood products, and serum hepatitis developed after being transferred to Walter Reed Army Medical Center.

serum hepatitis virus. The phenomenon of variation in virus strains is well known. Remarkable differences in mortality rates and duration of illness have been reported in different epidemics of infectious hepatitis. Although constitutional differences in the hosts undoubtedly influence the morbidity and mortality of this disease, it appears altogether likely that variation in virus strains must also play a part.

It also seems likely that unusual variants of the virus of infectious hepatitis may have a greater propensity for evoking the histologic pattern of cholestatic hepatitis. Not all the virus strains need necessarily damage the liver cells in identical manner. If crucial intracellular systems are blocked or destroyed, the liver cell will undergo necrosis. This is evidently what occurs in the classic type of viral hepatitis. In the cholestatic variety, however, there must be both quantitative and qualitative differences in the way the liver cell is damaged. Most of the parenchymal cells survive, even though some of the important functions are temporarily deranged. Perhaps in cholestatic hepatitis the secretory mechanism is more seriously disturbed as compared to other functions of the liver cell; perhaps there exists a disproportionate derangement of the mechanisms involved in the manufacture, secretion and propulsion of bile [*11,17*]. The view that such histologic variations in viral hepatitis arose from differences in virus strains was, of course, speculative [*18*].

COMMENTS

Terminology. The older term "cholangiolitic hepatitis" was introduced in an attempt to explain such intrinsic liver diseases that mimicked obstructive jaundice [*8*]. The concept behind this term was that the main lesion lay somewhere in the "cholangiole." Presumably this word "cholangiole" referred to the intralobular bile channel that runs from its origin at the central vein to the point where it empties into the interlobular bile duct in the portal tract. Although such a broad term (loosely analogous to the word "nephron") would be useful, it has been used so variously by different authors that it seems desirable to abandon it. Moreover, the term "cholangiolitic hepatitis" suggests that the lesion lies in the "cholangiole" and diverts attention away from other possible pathogenetic mechanisms, such as damage to the liver cell itself.

More recently other terms have been used which should wear better because they are purely descriptive and unprejudicial. The terms "cholestatic hepatitis" [*18*], "intrahepatic cholestasis" [*19,20*], and "primary intrahepatic cholestasis" [*21*] all highlight the main histologic feature, intrahepatic bile stasis, without committing one to a single pathogenetic mechanism. This is highly desirable, for it may turn out that the histologic picture of cholestatic hepatitis may result from several completely different mechanisms.

Viral Etiology of Cholestatic Hepatitis. Astute clinicians have long suspected that viral hepatitis may occasionally present the combined clinical and histologic aspects of cholestatic hepatitis [*7,8*]. This view is now being generally accepted [*10,12,18,20–27*], although some authors disagree [*28,29*]. This belief, however, cannot be proved conclusively without a specific laboratory test or transmission experiments in human volunteer subjects. Such data are still lacking. The best we can hope for under the circumstances is the kind of indirect evidence derived from epidemiologic data such as were afforded by our study.

In our series we were dealing with a disease presenting clinically as infectious viral hepatitis in a group of previously fit young men who had not been exposed to any known hepatotoxins or unusual environmental influences. This disease, which was centered around San Antonio, Texas, occurred in a minor epidemic form. Moreover, we were unable to isolate any pathogens from fresh liver tissue. Except for the unusually high incidence of cholestatic hepatitis (46 per cent), this disease would certainly be considered as infectious hepatitis. In the light of this background, we were led to the conclusion that we were probably dealing with some unusual strain of the infectious hepatitis virus. This view gained further support when Smetana reported his experiences with the Delhi epidemic of viral hepatitis that occurred during the winter of 1955 and 1956 [*12*]. Here again there occurred an epidemic of a disease clinically indistinguishable from infectious viral hepatitis which presented the histologic features of the "standard" histologic type in some cases and those of the "obstructive" type in others. In the Delhi epidemic the incidence of the latter type (cholestatic hepatitis) reached the remarkably high figure of 57 per cent in biopsied cases. These combined experiences strongly indicate, therefore, that

viral hepatitis may indeed cause cholestatic hepatitis. Furthermore, they suggest that what we call type A virus (or infectious hepatitis virus) is probably comprised of different substrains of virus.

The question arises as to why were not all cases in the Texas and Delhi epidemics of the the cholestatic variety? Here we would have to assume that certain quantitative or qualitative differences in liver cell damage were responsible for the variations in the histopathologic features. Perhaps this was partly influenced by host differences of which we were unaware. An analogy would be the remarkable variations in the histopathologic pictures evoked by the tubercle bacillus, depending upon such factors as dose and virulence of the organism, and the "immunity" state of the host, itself a balance of many complex factors. It may be that similar factors operate in viral hepatitis and indeed in other hepatocellular diseases [*18*].

Incidence of the Cholestatic Type of Viral Hepatitis. The incidence of the cholestatic type of viral hepatitis is roughly indicated by a study of the reports of biopsy cases submitted to the Registry of Hepatic Pathology by various military installations during the three-year period, 1951 through 1953. (Table VI.)* During this period 189 cases were submitted by the Far East Command; these cases occurred in our military personnel in Korea and Japan. Of these 189 cases only four (2.1 per cent) were of the cholestatic type. The European Command submitted 121 cases, of which four (3.3 per cent) were of the cholestatic type. Walter Reed Army Medical Center submitted seventy-seven cases of which six (7.8 per cent) were of the cholestatic type. By contrast, in the cases from Brooke Army Hospital studied during the same period the incidence of cholestatic hepatitis was much higher. Of fifty-two cases of hepatitis (both infectious hepatitis and serum hepatitis) occurring in Texas, twenty-one (40.4 per cent) were of the cholestatic variety; of thirty-eight cases stemming from the Far East, five (13.2 per cent) were of the cholestatic type.

When we take into account both the geographic residence and the immunologic type of hepatitis, we obtain interesting data. The case reports submitted by both the European and Far East Commands were almost exclusively those of infectious hepatitis; both groups showed a low incidence of the cholestatic type histologically (3.3 per cent and 2.1 per cent, respectively). The patients from Walter Reed Army Medical Center, which included a high proportion of serum hepatitis cases (at least 60 per cent), showed a higher incidence of cholestatic hepatitis histologically (7.8 per cent); unfortunately we do not have at this time a complete breakdown of the data which would enable us to compare the respective incidences of cholestatic hepatitis in the infectious hepatitis and serum hepatitis groups occurring at Walter Reed. Such data are available, however, from the Brooke Army Hospital series. (Table III.) Of fifty-two cases of hepatitis contracted in Texas, six were serum hepatitis and forty-six were infectious hepatitis. All six cases of serum hepatitis were of the classic histologic type, while of the forty-six cases of infectious hepatitis twenty-one (45.7 per cent) were of the cholestatic type. Of the thirty-eight cases of hepatitis contracted in the Far East, eight were infectious hepatitis and thirty were serum hepatitis. All eight cases of infectious hepatitis were of the classic histologic type, while of the thirty cases of serum hepatitis five (16.7 per cent) were of the cholestatic type.

Two other statistical features are noteworthy: (1) the higher incidence of cholestatic hepatitis in the Walter Reed series (which contained a high proportion of serum hepatitis cases) as compared to the relatively low incidence in the series from the European and Far East Commands, which were predominantly of the infectious type, and (2) the relatively high incidence of the cholestatic type in the group of serum hepatitis cases at Brooke Army Hospital, occurring in patients that had received blood products in the Far East.

There are at least two possible explanations for these data. One is that serum hepatitis virus is somewhat more apt to elicit the cholestatic reaction than is infectious hepatitis virus. On the other hand, if we assume that the "serum hepatitis" cases occurring in soldiers after transfer from Korea to Walter Reed and Brooke

* We assume that there was no special selection of cases that were to be studied by biopsy, and that the biopsy cases therefore constitute a reasonably accurate sample of the total populations with viral hepatitis. This assumption is probably correct, because during the time period under discussion viral hepatitis in our Armed Forces was under intensive study in various research centers and most of the biopsy case material submitted to the Registry came from these Viral Hepatitis Centers which were employing needle biopsy in randomly selected cases early in the disease.

Army Hospitals were actually cases of infectious hepatitis contracted in continental United States, then it would appear that the infectious hepatitis viruses prevalent in the United States were more apt to produce the cholestatic reaction than the infectious hepatitis viruses endemic in Europe and the Far East. Obviously no firm conclusion can be reached at this time. More data are needed based upon comparative epidemiologic studies in various parts of the world.

Histopathology of Viral Hepatitis. Evidently we must broaden our histopathologic criteria for the diagnosis of viral hepatitis to include the cholestatic type of reaction. As long as we acknowledged only the classic histologic type of viral hepatitis, the differential diagnosis was simple enough, for the histologic appearance of the classic variety is almost pathognomonic.* Fortunately, however, the cholestatic variety of viral hepatitis is uncommon. When such cases occur during an epidemic, the diagnostic problem becomes simpler. In sporadic cases, however, the diagnosis must always remain in doubt. Even after we have excluded other known causes of cholestatic hepatitis (such as drugs, peculiar hormonal states and leptospirosis) we can never be certain of the viral origin in individual cases as long as a specific laboratory test remains lacking.

The distinction from obstructive jaundice will be difficult in some cases and impossible in others. Liver function tests do not as a rule distinguish between obstructive jaundice and cholestatic hepatitis. In our limited experience the transaminase test is no exception in this regard [*31*]; this is to be expected, since this test reflects the degree of hepatocellular necrosis, which is slight in both entities. Nevertheless, if one waits four to six weeks before considering exploratory laparotomy in doubtful cases, as recommended currently by clinicians [*21,26*], the diagnosis will become clarified in most cases, either on clinical grounds alone or with the aid of needle biopsy of the liver. Needle biopsy specimens obtained four to six weeks after the onset of jaundice will permit histologic distinction between primary cholestatic hepatitis and obstructive jaundice in many cases. The greatest difficulty arises in cases of extrahepatic biliary obstruction in which the obstruction is incomplete or intermittent, such as is caused by calculi in the common bile duct or a sloughing tumor of the ampulla of Vater.

An interesting histologic feature of cholestatic hepatitis is the tendency for polymorphonuclear leukocytes to accumulate in the portal tracts and to a much lesser degree in the lobules, an uncommon finding in the classic variety. This leukocytic infiltration probably results from the chemotactic effect of bile. In obstructive jaundice (also characterized by intrahepatic bile stasis) the same phenomenon occurs. Indeed, in the latter condition one may often see tiny clusters of polymorphonuclear leukocytes surrounding the centrolobular bile plugs. It is our impression that these intralobular clusters are more common in obstructive jaundice than in cholestatic hepatitis. Why the leukocytic response should be more pronounced in the portal tracts remains a mystery. Apparently, both irritating substances and inflammatory cells drain from the lobules to the portal tracts [*10,32–34*], perhaps by way of the space of Disse. The more pronounced effect in the portal regions may be simply a matter of greater concentration of irritants and cells in these areas, or the result of some difference in reactivity between lobules and portal areas.

The degree of "ductular proliferation" in our cases of cholestatic hepatitis was generally slight. If anything, there was more of it in the classic variety. Certainly, it did not reach the degree generally seen in obstructive jaundice. The ubiquity of ductular proliferation in liver diseases again suggests that this may be the result of damage to liver cells produced by irritating substances draining to the periphery of the lobule [*33*]. Apparently the liver cells at the lobular periphery retain their embryologic capacity to form ductules. Preferential damage to liver cells at the periphery of lobules may again be the result of an increased concentration of irritants draining to this portion of the lobule, or of a greater susceptibility of these cells as compared to the cells in the centrolobular zones. Medium-sized and larger collecting bile ducts were normal. As indicated earlier, there was no clear-cut evidence that damage to the smaller interlobular bile ducts was a constant or significant feature.

Comparison of the Clinical Features of the Classic and Cholestatic Types of Viral Hepatitis. Laboratory tests: Our experience afforded a unique opportunity to compare the classic and cholestatic

* Even here our complacency has been shaken by the possibility that the drug iproniazid may produce a histologic reaction indistinguishable from the classic type of viral hepatitis [*30*].

varieties of viral hepatitis under conditions that were almost equivalent to a controlled experiment. The two groups showed no differences in the values obtained on admission of the total leukocyte count, the thymol turbidity test and the serum alkaline phosphatase test. The level of serum bilirubin (both the direct and total values) showed a correlation with the presence of intrahepatic bile stasis. Thus in patients with type B lesion (classic hepatitis with intrahepatic bile stasis) the mean value of serum bilirubin was twice that of those with A type lesion (classic hepatitis without cholestasis). The serum bilirubin level of patients with type C lesion (cholestatic hepatitis with focal parenchymal necrosis) was approximately equal to that of those with type B lesion in whom cholestasis also occurred. In the Delhi epidemic, Smetana [*12*] found no differences in the serum bilirubin values of the two histologic types; the thymol turbidity and flocculation values were slightly greater in the "standard" (classic) type of hepatitis. However, these tests were made only in a few cases and the significance of these data is uncertain.

Clinical course: The clinical appearance of patients was the same in both histologic groups. In both groups an occasional patient ran a more prolonged course, but there was no significant difference in the average duration of illness between the two groups. All patients recovered completely. In the Delhi epidemic, there was also no difference in the clinical aspects of the two histologic types. All stages of severity (from mild to fulminant) were found in both types. Three patients died of massive hepatic necrosis, but most patients recovered [*12*].

The findings in these two epidemics are somewhat contrary to general impressions held about cholestatic hepatitis, particularly in regard to duration of illness and the results of liver function tests. As regards the latter, it is generally believed that the serum alkaline phosphatase and total cholesterol levels should be higher, and the thymol turbidity and cephalin flocculation values lower, in the cholestatic type as compared to the classic type. This undoubtedly occurs in some cases, but it is precisely this type of "obstructive profile" which draws attention to these cases in the first place. Biopsy specimens are more apt to be obtained from patients with prolonged jaundice or from those with laboratory findings suggestive of the possibility of extrahepatic obstruction.

Another impression generally held is that the cholestatic type of viral hepatitis is more apt to run a prolonged course than the usual classic type. Here again this impression probably derives from a non-random selection of cases for biopsy. It is true that in occasional cases of the cholestatic variety the duration of illness is longer than usual, but this occurs in the classic type of viral hepatitis also. In both the Texas and Delhi epidemics there was no difference in the clinical duration between the two groups. Moreover, in our experience with a large group of viral hepatitis cases studied by biopsy in the Registry of Hepatic Pathology, we found many patients with the classic histologic variety who had also suffered a prolonged illness [*31*].

Relationship of Cholestatic Hepatitis to Primary Biliary Cirrhosis. Patients with cholestatic hepatitis, regardless of etiology, who run a protracted course could conceivably end up with the picture of "primary biliary cirrhosis" [*8,10,16,18, 21,29,35–38*].

One of us (I. N. D.) [*31*] saw a clear-cut example of progression to cirrhosis in a case of cholestatic hepatitis of unknown cause. The patient was a middle-aged man in whom an acute liver disease developed that was diagnosed clinically as "infectious viral hepatitis." The laboratory tests gave the following values: prothrombin time 62 per cent, thymol turbidity 5 units, total serum bilirubin 9.8 mg. per cent, alkaline phosphatase 19 Bodansky units, total cholesterol 360 mg. per cent. There was no history of exposure to drugs or hepatotoxins. Because of deepening jaundice, an exploratory laparotomy was performed three weeks after onset of jaundice. No obstruction of the biliary tree was found. The liver was grossly normal except for being bile-stained; a biopsy specimen showed a typical example of cholestatic hepatitis. After operation, jaundice continued and ascites developed, requiring paracenteses. The patient's general condition worsened and he died approximately three and a half months after the onset of his illness. At autopsy there was ascites and intense jaundice. No obstruction was found in the biliary system. The liver, which weighed 2,750 gm., showed a diffuse fine nodularity and cut with increased resistance. Histologically, the appearance was that of the early stages of a biliary cirrhosis; the sections showed centrolobular bile stasis, focal necrosis and inflammation of parenchyma, ductular proliferation, portal fibrosis with beginning

nodule formation, and infiltration of portal tracts with leukocytes.

Ricketts [*37*] has recently described similar cases. Moreover, in those cases of primary biliary cirrhosis that occur in middle-aged women, there are indications that in the earlier stages the histologic picture of cholestatic hepatitis is present [*38*].

Cholestatic hepatitis produced by drugs can also have a long duration in some cases [*10*]. One of us (I. N. D.) [*31*] saw an example of cholestatic hepatitis caused by penicillin allergy that lasted one and a half years before final recovery. He also studied a case of long-standing cholestatic hepatitis caused by the administration of arsphenamine. In the latter case the histologic features of the biopsy specimen taken at laparotomy six months after onset of jaundice were remarkably like those of a developing biliary cirrhosis. This patient also recovered clinically. We did not obtain a follow-up biopsy specimen in this case; presumably the liver lesion went on to healing with residual portal scarring that was insufficient to interfere with hepatic function.

It is becoming increasingly clear, therefore, that prolonged cases of cholestatic hepatitis, regardless of etiology, may go on to primary biliary cirrhosis. This is probably the kind of case that Hanot described [*39–41*], although the vagueness of his histologic descriptions must leave this controversial point unsettled [*8,29,34,35,42–44*].

Pathogenetic Mechanisms of Cholestatic Hepatitis. This subject has been hotly debated for some time [*7,8,10,11,17,19–21,24–29,34,35,38,45–50*]. The reviews on bile secretion by Brauer [*51*], and on biliary cirrhosis by Cameron [*34*] provide an excellent background for appreciating the problems we face in attempting to elucidate the pathogenetic mechanisms of cholestatic hepatitis.

Two central themes run through the hypotheses that have been suggested to explain the picture of cholestatic hepatitis: one is obstruction or damage to intrahepatic bile channels, and the other is hepatocellular damage leading to diminished bile secretion and bile propulsion by the liver cells. As long as thirty years ago Rössle [*35*] noted this division into two camps, for he pointed out that the pupils of Aschoff favored the cholangiolar hypothesis and the American authors (like Rous [*52*]) favored the hepatocellular theory. Heretofore, the former hypothesis has prevailed and scant attention has been paid to the possibility that hepatocellular damage of itself can lead to intrahepatic cholestasis in the absence of intrahepatic obstruction. Our analysis of the various mechanisms that have been proposed to explain intrahepatic cholestasis have been presented elsewhere [*11,17*]. In brief, we believe that hepatocellular damage will probably turn out to be the most important factor in the pathogenesis of intrahepatic bile stasis in the group of intrinsic liver diseases comprising the category of cholestatic hepatitis.

It may well be that what we see as the single histologic picture of cholestatic hepatitis is really a commonage produced by entirely different mechanisms in different cases, depending upon the etiologic agents. So far, the various views that have been expressed have been speculative, because there are few experimental data that bear directly on the problem. Nevertheless, the experimental data of Brauer [*51*] and Hanzon [*53,54*] certainly emphasize the indispensable role of the liver cell in bile secretion and bile flow; they could readily be taken to support the supremacy of the hepatocellular factor in intrahepatic cholestasis. It is obvious that a great deal of work is yet to be done on intrahepatic cholestasis before we can determine what similarities or differences exist in the pathogenetic mechanisms that are at play in such divergent conditions as viral hepatitis, chlorpromazine hepatitis, leptospirosis, neonatal bile stasis and many others.

Our present study does not provide a definitive answer. We were impressed by the fact, however, that when medium-sized or larger collecting bile ducts were present in our biopsy specimens, these were completely normal. We also noted that there was less ductular proliferation in the patients with cholestatic hepatitis than in those with the classic type. These observations tend to confirm our earlier impressions [*11,17*] that in cholestatic hepatitis hepatocellular damage probably plays the predominant role, and not "cholangiolar" damage or obstruction.

SUMMARY

During the period 1951 through 1953, 46 per cent of the cases of infectious hepatitis (studied by biopsy) occurring at Brooke Army Hospital, Texas, presented the histologic picture of cholestatic ("cholangiolitic") hepatitis. By contrast, during this same period, the patients at this hospital in whom serum hepatitis developed had a low incidence of cholestatic hepatitis, the majority showing the classic histologic picture

of viral hepatitis. An epidemiologic analysis of the patients with serum hepatitis showed that they were soldiers who had received blood products for battle injuries in Korea and had been immediately transferred to Brooke Army Hospital, where they came down with serum hepatitis after the usual incubation period. Thus the high incidence of cholestatic hepatitis occurred only among persons residing in Texas in whom the infectious type of viral hepatitis developed. In the control cases of viral hepatitis (both infectious and serum hepatitis) from other military hospitals in the United States, Europe and the Far East, that occurred during the same period (1951 to 1953), the patients showed a much lower incidence of the cholestatic variety, varying from 2 to 8 per cent.

Microbiologic studies on fresh liver tissue failed to yield a specific pathogenic agent. Nevertheless, after analyzing all the data at hand, we are inclined to believe that the hepatitis occurring in the Texas residents represented a minor epidemic of infectious viral hepatitis, and that the unusually high incidence of the cholestatic histologic variety was probably the result of some variant in virus strain. This suggests that the "virus of infectious hepatitis" is probably not a single virus but is comprised of a family of related substrains of virus. This may account in part for differences in morbidity and mortality rates heretofore reported, as well as for differences in the histologic pictures evoked by the viruses.

Our experience also indicates that we must broaden our histopathologic criteria for viral hepatitis to include not only the classic variety heretofore accepted but also the cholestatic type, and types intermediate between the two.

A comparison of the two groups of patients at Brooke Army Hospital, the one showing the classic histologic picture and the other the cholestatic type of viral hepatitis, revealed no significant differences in (1) the clinical appearance, (2) the duration of illness, (3) the final outcome (all recovered) and (4) the initial values for total leukocyte count, the thymol turbidity test and the serum alkaline phosphatase. However, the mean value for serum bilirubin (both direct and total) was twice as high in the groups of patients showing intrahepatic bile stasis as in those in whom biopsy specimens were free of intrahepatic cholestasis. This indicated a correlation between the presence of intrahepatic cholestasis and the level of serum bilirubin.

The intrahepatic bile ducts showed no lesions. For this and other reasons we favor the view that the predominant pathogenetic mechanism operating in cholestatic hepatitis is hepatocellular damage and not injury or obstruction to intrahepatic bile channels. It seems likely that hepatocellular damage, even without obvious necrosis, can lead to impairment of manufacture, secretion and propulsion of bile.

Acknowledgment: The statistical analyses were kindly performed by Harold Balaban and Allen M. Singer, both of the Department of Epidemiology, Walter Reed Army Institute of Research, Walter Reed Army Medical Center. The statistics on viral hepatitis were graciously supplied by Mrs. Mardelle Reynolds and others of the American Registry of Pathology, Armed Forces Institute of Pathology.

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Primary Mediastinal Echinococcosis*

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PRIMARY mediastinal echinococcosis, i.e., echinococcosis of the mediastinal supporting tissue arising directly from ingested ova, is considered an extremely rare condition. The eminent hydatidologist Dévé who, in the course of fifty years published 325 papers on hydatid disease, even contested a primary mediastinal location [*69,70*]. However, the recent development of mass roentgenography and thoracic surgery in countries in which echinococcosis is endemic has shown that mediastinal hydatid disease is not as uncommon as believed.

In the collective statistics dealing with echinococcosis in all sites, the occurrence of mediastinal hydatid disease varies from zero to 6 per cent. (Table I.) The first case of mediastinal echinococcosis, reported by Chaussier [*15*] in 1807, was a cyst of the posterior mediastinum encroaching upon the spinal cord, resulting in paraplegia and disturbance of the sphincters. It was discovered at autopsy in a young woman who died after a painless childbirth. The second case, reported in 1829 by Alibert [*2*], was a giant cyst in the anterior mediastinum which had perforated the pericardium and caused death by tamponade. In the literature we found sixty-two reports on mediastinal echinococcosis comprising altogether seventy-four cases [*1–62*]; all but six of these papers [*17,18,26,30,31,51*] reported single cases only. The only review of mediastinal echinococcosis (Monod and Weil) was based on nine cases, eight of them collected from the literature [*41*]. The purpose of this paper is to present six additional cases of mediastinal echinococcosis and to review this disease in the light of information which has recently become available.

CASE I. *Echinococcus cyst of the posterior mediastinum with intraspinal extension; compression of the spinal cord causing paraplegia and paralysis of the bladder:* P. Z., a thirty year old clerk born and bred in Israel, was reported to be healthy until 1942, when he felt pains in the back which persisted for ten days. In 1944 the backache reappeared, radiating to both sides of the

TABLE I

MEDIASTINAL ECHINOCOCCOSIS

Series and Authors	Country	Echinococcosis (all sites)	Mediastinal Echinococcosis	
		No. Patients	No. Patients	Per cent
1, Dévé [*68*]	Various countries	2,727	0	0
2, Barnett [*63*]	Australia	1,732	0	0
3, Giromini, Granati [*76*]	Italy	5,048	0	0
4, Ferro et al. [*71*]	Argentine	7,740	0	0
5, Magath [*79*]	U.S.A. and Canada	491	0	0
6, Geroulanos [*75*]	Greece	4,325	7	0.16
7, Daniliak [*19*]	U.S.S.R.	445	1	0.22
8, Colombani [*67*]	Austria	147	2	1.35
9, Gajardo-Tobar, Zenteno [*73*]	Chile	114	2	1.75
10, Ugon [*85*]	Uruguay	283	5	1.77
11, Carmalt-Jones [*66*]	New Zealand	90	2	2.2
12, Present report	Israel	100	6	6

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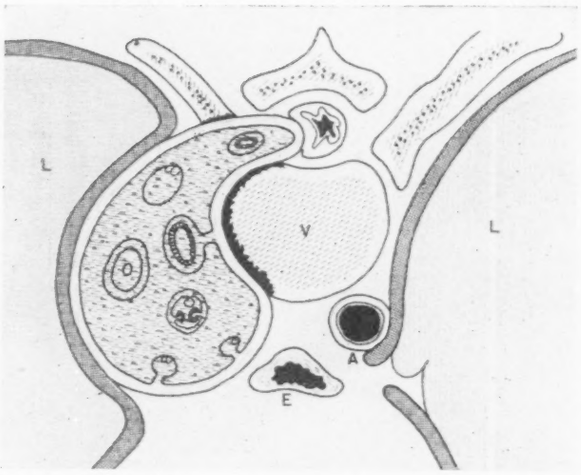


FIG. 1. Case I. Echinococcus cyst of the posterior mediastinum with spinal extension and compression of the spinal cord.

chest. Roentgenogram of the chest showed a round opacity located in the right paravertebral area between the seventh and eighth ribs. The seventh right intercostal space was enlarged and there were osteolytic changes of the lower edge of the seventh rib and of the upper edge of the eighth rib near the costovertebral articulations. A few days later the patient complained of weakness in the left leg which gradually increased and later involved the right leg also. On admission to the hospital on January 1, 1945, he was almost totally paraplegic with complete paralysis of the bladder. Myelography showed a complete block at the level of D6 to D7. Sensitivity to touch was normal. Sensitivity to pain and warmth and deep sensitivity were decreased below the level of D6.

Laminectomy performed on January 4, 1945 exposed a large extradural cyst. It was a dumb-bell type of cyst which passed from the anterior surface of the dura to the seventh and eighth intercostal spaces, with projection into the posterior mediastinum. (Fig. 1.) The seventh intercostal space was about three times its normal width; the seventh and eighth ribs as well as the lateral surface of the corresponding vertebrae were eroded. The cyst was aspirated and incised, and clear fluid including a number of small vesicles was evacuated. The extradural portion of the cyst, as well as the endocyst with its contents, were removed. Ten days later, through a right posterolateral thoracotomy, the fibrous pericyst which extended deep into the posterior mediastinum was excised. This was followed by slight improvement of motor function and sensitivity of the legs. In November 1945 the patient was readmitted to the hospital in poor general condition with spastic paraplegia, urinary retention and severe constipation. Myelography showed free passage of lipiodol. He died three months later from an infection of the urinary tract.

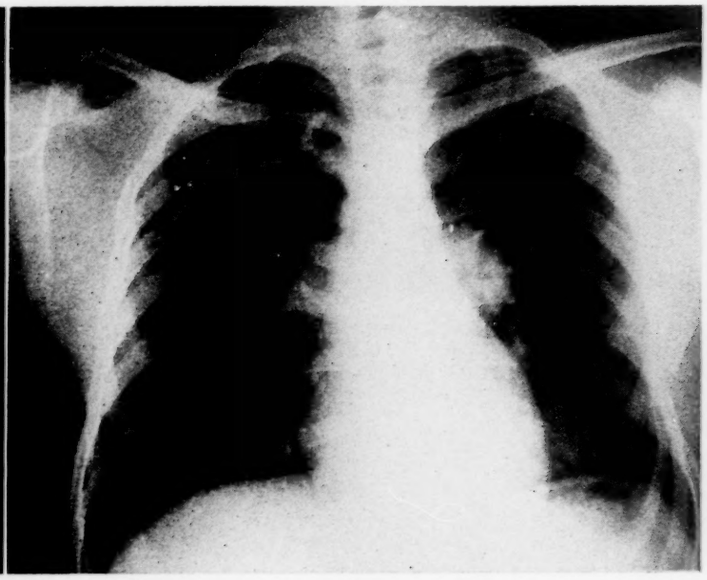
CASE II. *Echinococcus cyst of the posterior mediastinum eroding vertebrae and ribs:* H. J., a fifteen year old boy, immigrated from Persia to Israel in 1951. He had worked as a herdsman and had had a dog which died from an unknown cause. Routine mass roentgenography in March 1956 disclosed a round shadow in his left hemithorax. On admission to the hospital in August 1956 he was asymptomatic, except for a circumscribed area of hypersensitivity at the level of the seventh rib. No tubercle bacilli were found in the sputum and gastric washings. Results of the Casoni and Weinberg tests were negative; the blood count yielded normal values with 2 per cent eosinophils. Further roentgenographic studies showed the round shadow located in the left paravertebral gutter about the seventh intercostal space; there was osteolysis of the head of the seventh rib, the upper edge of the eighth rib and the lateral aspect of the adjacent vertebral body. (Fig. 2.) On fluoroscopy the shadow did not move during respiration.

On August 17, 1956, left posterolateral thoracotomy was performed. The lower lobe of the left lung was adherent in the region of the tumor, which was located in the seventh intercostal space, was 3 by 5 cm. in diameter, covered by parietal pleura and bulging into the mediastinum. After evacuation of the multivesicular endocyst and resection of the pericyst, the destruction of the lateral part of the vertebral body was visualized. There was no intraspinal extension of the cyst although the cystic wall was in direct contact with the dura. The postoperative course was uneventful and the boy left the hospital on the tenth day after the operation.

Two years later, on routine roentgenologic examination, bilateral round opacities were found in both parahilar areas. (Fig. 3.) The patient was readmitted to the hospital in August 1958, with the diagnosis of recurrent bilateral mediastinal echinococcosis. Further roentgenographic studies, including tomography, revealed that the recurrent hydatids of the left and right posterior mediastinum were apparently in direct communication through the prevertebral space. This had been widened by erosion of vertebral bodies D7 and D8, and by elevation of the prevertebral fascia. (Fig. 4.) This assumption was confirmed at operation, which was performed in two separate stages. On August 14, 1958, the left side of the chest was reopened through the bed of the sixth rib, and a cyst measuring about 8 cm. in diameter was exposed in the posterior mediastinum. The anteromedial wall of the cyst was densely adherent to the aorta and could not be separated from it. (Fig. 5.) The pericyst was then opened, the fluid aspirated, and the parasitic membrane removed. Thereafter an eroded area in the aortic adventitia measuring 3 by 2 cm. became visible. This was covered by suture of the redundant edges of the fibrous pericyst over the involved area. On October 2, 1958, a right thoracotomy was performed through the bed of the seventh



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FIG. 2. Case II. Echinococcus cyst of the posterior mediastinum. Tomogram in oblique position showing marked widening of the intercostal space in the vicinity of the cyst.

FIG. 3. Case II. Recurring echinococcus cyst of the posterior mediastinum located in both paravertebral gutters.

rib. A cyst measuring 6 cm. in diameter was exposed and the multivesicular endocyst removed. The erosion of vertebral bodies D7 and D8 was evident also from this approach and the communication with the left mediastinum was visualized clearly.

The patient's recovery after both operative stages was entirely satisfactory, and he left the hospital on October 13, 1958.

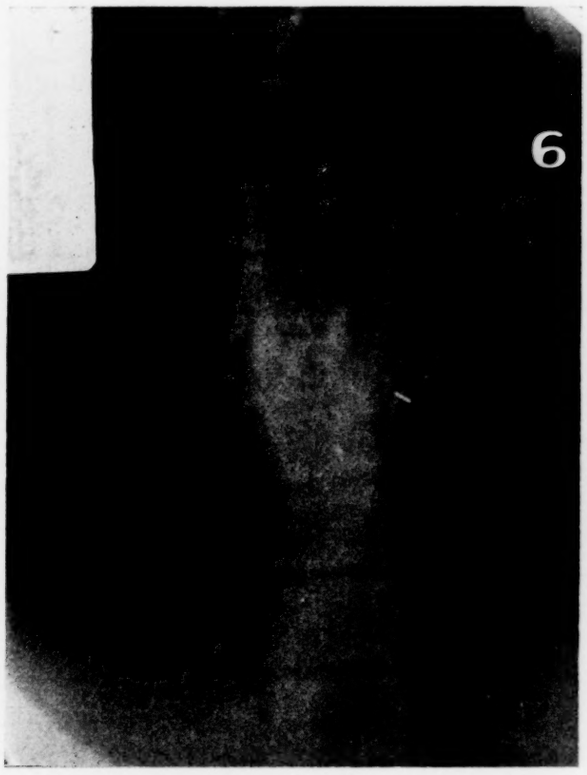


FIG. 4. Case II. Anteroposterior tomogram showing pressure necrosis of the eighth vertebral body.

CASE III. *Echinococcus cyst of posterior mediastinum eroding ribs and penetrating the posterior thoracic wall:* S. R., a thirty year old woman, had come to Israel from Iraq in 1950. Her present illness began in 1951 when a circumscribed swelling appeared in the region of the left scapula. When the swelling increased and became painful it was incised in an outpatient clinic, apparently having been diagnosed as an abscess. In

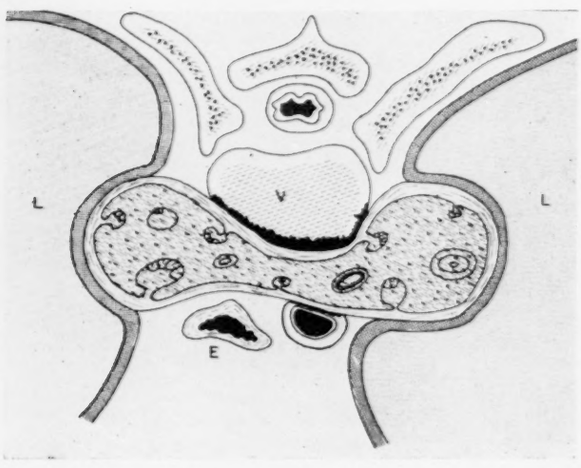


FIG. 5. Case II. "Pince-nez" echinococcus cyst of the posterior mediastinum.

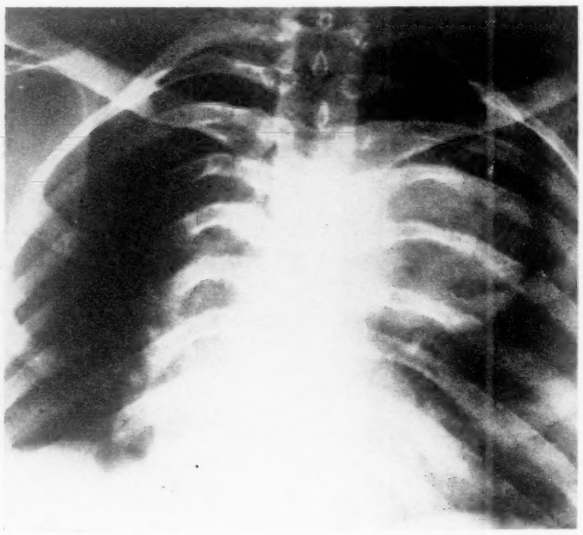


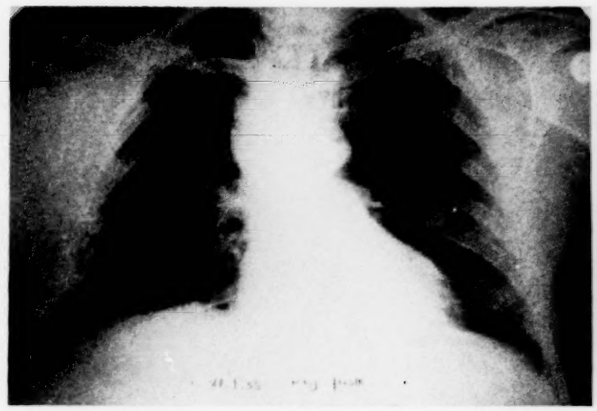
FIG. 6. Case III. Echinococcus cyst of the posterior mediastinum, with pressure erosion of the fourth, fifth and sixth ribs, and widening of the corresponding intercostal space.

May 1955 the swelling reappeared and the patient was operated on in another hospital at which time a clear, watery fluid was aspirated from the cyst; the cytologic examination of this fluid revealed scolices. No attempt was made to remove the ectocyst and the swelling recurred. In September 1956 the patient was admitted to the hospital, complaining of a nonproductive cough and pain in the left posterior wall of the chest. Physical examination revealed a sensitive bulging area overlying the posterior portion of the left, fourth, fifth and sixth ribs. Result of the Casoni test was positive; result of the Weinberg test was negative. Roentgenography showed a round paramediastinal opacity measuring 4 cm. in diameter in the left side of the chest, and osteolytic changes in the posterior parts of the fourth, fifth and sixth ribs. (Fig. 6.) Operation was performed on September 30, 1956. Through a left posterolateral thoracotomy the posterior mediastinum was entered, and an echinococcus cyst the size of an egg was exposed. The cyst extended from the fourth, fifth and sixth ribs posteriorly to the posterolateral aspect of the thoracic aorta. The cyst was full of pus and daughter cysts; its contents were evacuated and the fibrous ectocyst, except that part which was already adherent to the mediastinal pleura and the aortic wall, was resected. The necrotic parts of the fourth and sixth ribs were also resected and the remaining space was formalized and drained. Histologic examination of the resected ribs showed advanced necrosis but no infiltration of hydatid microvesicles. The postoperative course was uneventful and the patient made a complete recovery.

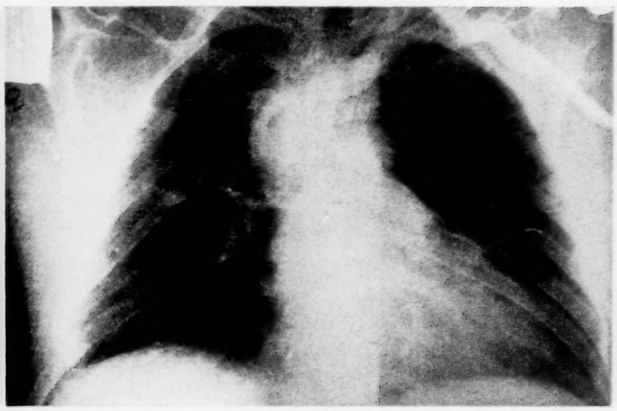
CASE IV. *Echinococcus cyst of the middle mediastinum compressing the vena cava superior and eroding the aortic wall:* O. M., a forty-four year old fisherman, immigrated to Israel from Morocco in January 1958. In April 1954 he was operated on because of a pulmonary hydatid cyst in the upper lobe of the right lung; two months later an echinococcus cyst of the left lower lobe was removed. Two years later he was operated on again because of recurrence of the hydatid cyst in the upper lobe of the right lung. In January 1958, at routine roentgenography after his arrival in Israel, a round, sharply defined opacity was discovered in the superomedial part of the right hemithorax. (Fig. 7.) The patient's only complaint was a sensation of pressure in the sternal area. Results of the Casoni and Weinberg tests were positive. On fluoroscopy, the mass did not pulsate and did not move with swallowing. Phlebographic studies showed dilatation of the left innominate vein in its distal part and narrowing in its proximal part; the superior vena cava also was narrowed, and both veins were markedly displaced right and upward skirting the round mass in the right upper mediastinum. (Fig. 8.)

At operation, through a right posterolateral thoracotomy, an echinococcus cyst the size of an orange was exposed, extending from the second to the fifth ribs laterally and from the trachea medially into the anterior mediastinum. The cyst caused marked displacement of the superior vena cava; its medial limits were impossible to define. The cyst was aspirated and clear fluid was removed. After evacuation of the parasitic membranes massive hemorrhage suddenly occurred. This was seen to originate from an erosion of the aortic arch proximal to the origin of the right innominate artery. The aortic wall had been damaged in this area by long-continued hydraulic pressure from the cyst and the resultant friability of the wall of the vessel made repair of the defect extremely difficult. The patient lost about 6 L. of blood which was rapidly restored by transfusion. The postoperative course was complicated by a right clotted hemothorax, which had to be evacuated later by a small anterior thoracotomy. Thereafter his recovery was undisturbed.

CASE V. *Echinococcus cysts of the anterior mediastinum and heart:* M. N., a twenty-nine year old male bookbinder, immigrated to Israel from Iraq in 1951. His present illness began in July 1957 with palpitations and a sensation of retrosternal pressure. He also complained of progressive weakness. He had lost 6 kg. in weight. On admission to the hospital in April 1958, physical examination revealed a soft systolic murmur at the left sternal border and dullness in the superior retrosternal area. The roentgenographic examination, including tomography, showed a well defined opacity in the left anterior mediastinum and a small round shadow near the border of the left side of the heart. (Figs. 9 and 10.) The electrocardiogram showed marked right axis shift, inverted T waves in leads II III, V_4, V_5 and V_6, P waves peaked in leads II and III, and ventricular premature beats. Result of the Casoni



7



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FIG. 7. Case IV. Echinococcus cyst of the middle mediastinum (para-aortic). Posteroanterior view.

FIG. 8. Case IV. Angiogram showing marked narrowing and displacement of the superior vena cava and the innominate vein by the hydatid cyst.

test was positive, result of the Weinberg test was negative. Other laboratory tests gave normal results.

At operation, a left anterolateral thoracotomy through the fourth intercostal space was performed and in the anterosuperior mediastinum an echinococcus cyst the size of a man's fist was exposed. The cyst was aspirated of its fluid contents and the parasitic membrane was then removed *in toto*. The ectocyst was cut away and the mediastinal pleura left open for drainage. The anteroinferior surface of the pericardium was thickened and a multicystic fluctuating mass protruded from this region. When the pericardium was opened it became clear that it had been penetrated by a multivesicular echinococcus cyst originating from the left ventricular myocardium. The affected area of the ventricular wall, which measured about 5 by 6 cm., was fully exposed and by incision of the anterior layer of the pericyst a large number of daughter cysts of different size were evacuated. The resulting defect in the anterior ventricular wall was closed by mattress sutures approximating the opposite margins of the fibrous pericyst.

The postoperative course was uneventful. The palpitations, sensations of pressure and systolic murmur disappeared. The patient left the hospital two weeks after operation. At follow-up examinations he was found to be in excellent health. His electrocardiogram was normal.



FIG. 9. Case V. Echinococcus cyst of the anterior mediastinum. Posteroanterior view.



FIG. 10. Case V. Echinococcus cyst of the anterior mediastinum. Right oblique view.



FIG. 11. Case VI. Echinococcus cyst of the anterior mediastinum. Posteroanterior view.

CASE VI. *Suppurated echinococcus cyst of the anterosuperior mediastinum adherent to the subclavian vessels:* A. S., a twenty-three year old man, immigrated to Israel from Morocco in 1955. His present illness was discovered in March 1956 at mass roentgenography, which disclosed a round shadow in the upper mediastinal part of the left hemithorax. (Figs. 11 and 12.) One year later roentgenographic examination showed that this shadow had increased. The Casoni test gave a positive result, and a blood count revealed 8 per cent eosinophilia. The patient was admitted to the hospital and on May 5, 1957, a left posterolateral thoracotomy was performed. A fluctuant cyst the size of an egg, with thick walls, was exposed in the upper mediastinum. It was adherent to the subclavian vessels and the apicomediastinal surface of the lung. After dissection of the cyst from the lung it was opened and necrotic membranes with thick pus and multiple daughter cysts were removed. The pericyst also was resected, except that part which was closely adherent to the subclavian vessels. The postoperative course was uneventful and the patient left the hospital two weeks after operation.

COMMENTS

Pathogenesis. All statistics dealing with the location of hydatid disease in man show that 15 to 20 per cent of the hexacanthe embryos succeed in passing the hepatic and pulmonary capillary barrier and reach the systemic circulation.

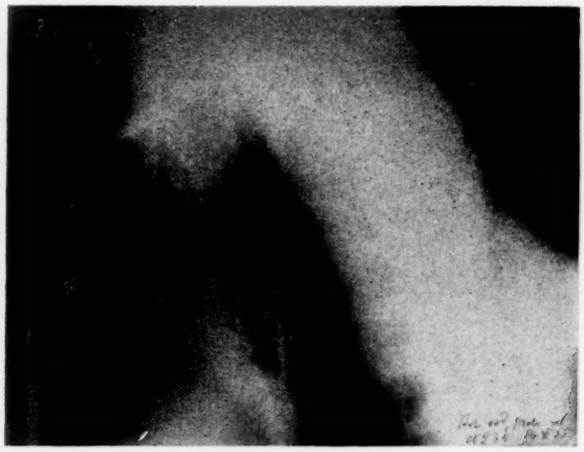


FIG. 12. Case VI. Echinococcus cyst of the anterior mediastinum. Lateral tomogram.

Hydatid cysts consequently may be found in every tissue and organ, without any particular site of election. However, the more vascularized the tissue or organ, the greater are the chances of implantation of the hexacanthe embryo. The wide-meshed subpleural plexus of Turner, formed by anastomoses of the internal mammary artery with the posterior intercostal and bronchial arteries, may represent the terminus of a trip of the six-thorned embryo, which is of the size of a leukocyte. These cysts arising in the immediate vicinity of the mediastinal pleura, from the subpleural [*14*], peripleural [*9*] space, are the most frequent of all primary mediastinal cysts. The hematogenous pattern in the pathogenesis of the mediastinal echinococcosis was demonstrated experimentally in a squirrel by Dévé [*68*].

Clinical and Pathologic Aspects. The age of the eighty patients with mediastinal echinococcosis collected from the literature (including our own patients) varied from eight to fifty-seven years. The disease was more frequent in men than in women, the ratio of incidence being 6:4. There was equal distribution of the disease on both sides of the mediastinum. Almost all posterior mediastinal hydatid cysts were multivesicular. However, in the middle mediastinum almost all were univesicular. These differences can be explained by the fact that daughter cysts are produced in the mother cysts when the development of the parasite is threatened by encountering hard obstacles, such as the bony structure in the posterior mediastinum.

The clinical aspects of mediastinal echinococcosis vary according to the location.

The posterior mediastinum is the most common site of occurrence of mediastinal hydatid disease. Of eighty primary mediastinal cysts, forty-four (55 per cent) were located in this region. Most of these cysts originated in the paravertebral gutter. Anatomically, the paravertebral gutter is not a part of the true mediastinum. However, for clinical purposes, all lesions located there are commonly included in the pathology of the posterior mediastinum. Small cysts are frequently asymptomatic or cause only few symptoms. They are usually found at routine roentgenography and diagnosed only at the time of surgical intervention (Case II). They may produce erosion of ribs and vertebrae due to long-continued pressure, exposing the meninges (Cases I and III).

The transverse processes, laminas, pedicles and lateral aspect of the body are the parts of the vertebrae commonly affected. The intervertebral discs and ligaments are left intact. The neighboring eroded ribs are widely separated from each other. Widening of the intercostal spaces and of the intervertebral foramina is an important roentgenologic sign. (Figs. 3 and 6.) The spinal cord may be compressed laterally, or more frequently from its anterior aspect. The most frequent clinical picture in these patients is paraplegia, sometimes hemiplegia [*34*]. Twenty-five cases of compression of spinal cord by mediastinal hydatid cysts have been reported. Today these cases are certainly less frequent, due to earlier surgical intervention.

Other hydatid cysts extend from the paravertebral gutter to the posterior thoracic wall (Case III). In such cases the course of the disease may be very long. In a case reported by Donoso and Steimle [*26*] the paravertebral cyst, bulging outward, formed a tumor 10 by 20 cm. in diameter, which was diagnosed as a lipoma. After fifteen years this tumor compressed the spinal cord, causing paralysis of the lower extremities, and was diagnosed only at operation as an enormous hydatid cyst. Extending upward and outward, the hydatid cysts may compress the neurovascular structures, causing the development of Pancoast's syndrome, cubital paralysis, superior vena cava syndrome [*31*], Horner's syndrome [*62*] or compression of the subclavian artery [*31*]. Other hydatid cysts of the posterior mediastinum may originate anterior to the vertebral column, between the aorta and esophagus, bulging towards either pleura [*51*] or perforate in the pleural space [*46*]. A special form of the posterior mediastinal hydatid disease is the "pince-nez" echinococcus cyst occupying both paravertebral gutters with prevertebral communication. (Fig. 5.)

Hydatid Cysts of the Middle Mediastinum. These are extremely rare and only six cases have been reported in the literature. Their location was between the great vessels at the base of the heart [*52*], under the aortic arch [*29*] or between the diaphragm, pericardium and the middle lobe [*31*]. These cysts produce symptoms of precordial pain, dyspnea and cyanosis. Roentgenography and fluoroscopy in different projections are necessary to demonstrate these cysts; phlebography or angiocardiography may be valuable in showing compression of the great vessels. (Fig. 10.) The danger of pressure erosion of these vessels, with fatal hemorrhage, is serious.

Echinococcosis of the anterior mediastinum was found in 36 per cent of all patients with hydatid cysts. The hydatid cysts of the anterosuperior mediastinum constitute a distinctive group. Compression of the trachea with dyspnea frequently is the presenting syndrome [*27,35*]; in four cases it led to fatal strangulation [*1,23,53,58*]. An expanding cyst may also compress the neurovascular structure, causing the superior vena caval syndrome [*23,27*] or Horner's syndrome [*23*]. Located behind the sternum and extending upward they may be palpable above the manubrium [*25*] or they can even extend to the neck [*10,39,44*] and perforate the subclavian artery [*24*]. An anteriorly extending cyst can be palpable in the parasternal area [*19,40*] and produce erosion of the sternoclavicular joint [*31*].

Hydatid cysts of the anteroinferior mediastinum expanding toward the lungs do not cause symptoms for a long time [*37,52*] and may attain enormous dimensions, occupying almost the entire hemithorax [*8*]. Pressing on the heart, they produce precordial pain and paroxysmal vertigo [*8*]. They may perforate the pericardium, causing fatal cardiac tamponade [*2,45*].

Diagnosis. Mediastinal hydatid disease should be considered in every case of mediastinal cyst or tumor, particularly in countries with endemic echinococcosis. In the available statistics on 1,302 surgical mediastinal tumors and cysts, mostly from countries without endemic echinococcosis, we did not find mention of a single hydatid cyst. However, in our own material, during the last five years, of thirty-five mediastinal lesions surgically treated five were hydatid cysts (14.3 per cent).

The differentiation of mediastinal hydatid cysts from other mediastinal lesions may present difficulties. In the posterior mediastinum they may simulate neurogenic tumors which, located in the paravertebral gutter, are known to erode the vertebrae and ribs in the same manner and also grow in "hour-glass" fashion. The statement that tumors of the paravertebral gutter are neurogenic until proved otherwise holds true only for countries without endemic echinococcosis. Meningocele also may closely simulate posterior mediastinal hydatid cyst. Recklinghausen's disease, which accompanies meningocele in 60 per cent of cases [*84*], may be helpful in the differential diagnosis.

In the middle mediastinum hydatid cysts should be differentiated from bronchogenic cysts, gastroenterogenous cysts, aneurysms of the great vessels and localized Hodgkin's lymphadenopathy. In the anterosuperior mediastinum they should be differentiated from intrathoracic goiter, thymoma and dermoid cyst; in the anteroinferior compartment from pericardial cyst, lymphangioma and Morgagni's hernia.

Casoni's intradermal test and Weinberg's complement fixation test are sometimes helpful in establishing the diagnosis. In our patients with echinococcosis of all sites results of the Casoni test were positive in 78 per cent. However, only a strong reaction, with erythema and edema occupying the entire forearm, arising immediately and persisting some days, was convincing proof of echinococcus disease. This was encountered in only 20 per cent of the patients. A reaction of 2 to 3 cm. in diameter, seen mostly in our patients with echinococcosis, was encountered frequently also in patients without echinococcosis. A false positive Weinberg reaction was encountered even more frequently. Eosinophilia (above 7 per cent) was noted in only 25 per cent of the patients with echinococcosis.

Primary mediastinal echinococcosis should be differentiated also from ossifluent hydatid abscess issuing from affected vertebrae into the mediastinum. Called hydatid Pott's disease [*78*], this form of echinococcosis may be recognized on routine roentgenography, which reveals a paravertebral opacity with irregular, sometimes calcified outline. A single, localized lesion is rare; the anterior mediastinal pocket may communicate with intraspinal or posterior pockets. The lesions of osseous structures are roentgenologically similar to lesions caused by pressure. At surgical exploration the hydatid abscess is easily recognized. Osseous echinococcosis is always of the multimicrovesicular type; there are no proper hydatid cysts and no membranes. The bone is infiltrated by scattered microvesicles, and puncture of the mediastinal pocket reveals no fluid, the microvesicles being immersed in a thick purulent magma. However, in old suppurated cysts, after necrosis of the membranes and infiltration of the neighboring bones by vesicles, localization of the primary site of echinococcosis is frequently impossible.

Cardiopericardial echinococcosis is another important localization of mediastinal hydatid disease, and should be differentiated from primary mediastinal echinococcosis. A cardiac cyst may be suspected when roentgenograms show a round prominence in the cardiac shadow and the electrocardiogram is abnormal. Pericardial hydatid cysts are usually multiple and may produce multiple prominences on the cardiac outline. They may reach the size of an orange and bulge even into the superior mediastinum [*77*]. The true origin of these juxtapericardial cysts may be difficult to determine. A hydatid cyst closely adherent to the pericardium, with signs of obliterative pericarditis, is probably of myocardial origin. Even at operation it may be difficult to find the scar of the ruptured myocardial cyst because of its superficial location and the pericardial fibrosis present [*80*].

Paramediastinal echinococcosis may also present difficult diagnostic problems. The most frequent form of paramediastinal echinococcosis is the pulmonary mediastinal form [*72,74,81*]. The hydatid cyst arises in the peripheral pulmonary tissues, bulges from the mediastinal surface of the lung into the mediastinum, and may closely simulate a primary mediastinal cyst. However, in paramediastinal echinococcosis a history of hemoptysis is not uncommon; pulmonary cysts do not erode vertebrae and ribs, although compression of the esophagus and the superior vena cava may occur [*82*]. The diagnosis is frequently made only at operation when bronchial openings are found in the pericyst.

Phrenomediastinal echinococcosis is the designation for hydatid cysts arising in the diaphragm [*31*] or in the subphrenic space [*83*] and expanding upward toward the mediastinum. In parietomediastinal echinococcosis, cysts arising in the muscles of the thoracic wall bulge into the posterior mediastinum, extending also into the epidural space [*5*].

Secondary mediastinal echinococcosis results from rupture of paramediastinal or pericardial cysts into the mediastinum or from implantation of the scolices when the hydatid fluid leaks out during surgical intervention. Secondary echinococcosis resulting from rupture of the mother cyst is always multiple. The diagnosis is evident when multiple cysts fill the mediastinum and adhere to all its organs [*64,65*].

Treatment. Surgery is the treatment of choice and the only means of establishing the final diagnosis. A posterolateral thoracotomy provides adequate exposure for nearly all mediastinal cysts. Cysts situated in the superior and anterior mediastinum may be approached by anterolateral thoracotomy or by partial sternotomy. When extension of a posterior cyst into the spinal canal occurs, laminectomy is necessary and the intraspinal portion of the cyst should be removed first.

Theoretically, the ideal operation would encompass complete extirpation of the parasite with its pericyst. However, removal of the latter is unnecessary and may even be hazardous owing to dense adhesions to vital mediastinal structures. Therefore, it is sufficient to remove the parasite cyst with all its contents, taking care to avoid spilling. The redundant part of the pericyst is then excised and the remaining cavity painted with formalin.

SUMMARY

The clinical and pathologic features of mediastinal echinococcosis are discussed in the light of six cases herein presented and a review of seventy-four cases reported in the literature.

In our patients a mediastinal location occurred in 6 per cent of echinococcosis in all sites. The frequency of subpleural-mediastinal location and the role of the subpleural plexus in the pathogenesis of mediastinal echinococcosis is emphasized.

Mediastinal hydatid cysts are usually multivesicular, except for those in the middle mediastinum.

Fifty-five per cent of all cases of mediastinal hydatid cysts were located in the posterior mediastinum. Erosion of the ribs and vertebrae and compression of the spinal cord occurred in 57 per cent of all posteromediastinal hydatid cysts. In 36 per cent of cases the mediastinal echinococcosis was located in the anterior mediastinum; compression of trachea with severe dyspnea or strangulation was the major complication. In echinococcosis of the middle mediastinum (9 per cent of all cases), compression and erosion of the great vessels is the main danger.

Briefly summarized is the differential diagnosis of mediastinal echinococcosis from other mediastinal cysts and tumors, as well as from mediastinal ossifluent hydatid abscess arising from vertebral echinococcosis, from cardiopericardial hydatid disease and from paramediastinal echinococcosis.

Various approaches in the surgical treatment of mediastinal echinococcosis are discussed.

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Course and Prognosis of Sarcoidosis*

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SOME investigators [*1,2*] have emphasized the benign course of sarcoidosis and its low mortality, while others [*3,4*] have called attention to the frequency of disability and of death as a result of this disease. Disparate views on the course of sarcoidosis may result from differences in definition of the disease, in the race of patients, and in length of observation. Differences result from selection of patients through their referral from hospital wards or from dermatologic, ophthalmologic or pulmonary clinics, and greater exposure to tuberculous infection in some populations may also contribute to the variability of clinical experience in sarcoidosis.

The observations on the course of disease in 211 patients with sarcoidosis, presented herewith, are of interest for several reasons. Few reports of prolonged observation of large numbers of patients have appeared and data are needed by which prognosis can be estimated and treatment evaluated. Comparison of the course of sarcoidosis in Europe, the United States and other countries is important to ascertain whether or not sarcoidosis is a disease entity essentially similar in various parts of the world. Opinions still differ as to the frequency with which sarcoidosis and tuberculosis are associated. Finally, study of the natural history of this disease may provide clues to the nature of sarcoidosis.

CLINICAL MATERIAL

The 211 subjects included in this study are comprised of patients diagnosed between 1938 and 1958 as having sarcoidosis, and followed up for at least three months at the Sarcoid Clinic of the Henry Phipps Institute, the Graduate Hospital of the University of Pennsylvania or the Woman's Medical College of Pennsylvania. Ninety-two patients (43.6 per cent) were referred from outpatient clinics or photofluorographic units. Eighty-five patients (40.3 per cent) were referred to the Sarcoid Clinic after having initially been studied in general hospitals.‡ Thirty-four patients (16.1 per cent) were referred by private physicians. Involvement of the respiratory tract led to the study of 160 patients; cutaneous manifestations led to referral in twenty-one instances and ocular lesions in fifteen instances. Miscellaneous lesions accounted for the remainder of the referrals.

Clinical manifestations were absent or mild in many instances, but some patients were seriously ill with involvement of lungs, eyes, kidneys or heart. One or more biopsy specimens showing epithelioid cell granulomas consistent with sarcoidosis were obtained from all patients. Every effort was made by appropriate studies to exclude tuberculosis, fungal diseases and beryllosis. The clinical manifestations encountered in the first 160 of these patients have been described previously [*5*]. It is believed that the material presented herein is representative of sarcoidosis in a large American city, and is not weighted by unduly large numbers of patients with any one type of organ involvement. (Table I.)

The duration of observation was dated from the time objective evidence of sarcoidosis was first demonstrated, either by roentgenographic changes or by histologic evidence of epithelioid tubercles. The period of observation was terminated on May 31, 1958. After each examination the status was estimated on the basis of clinical manifestations and roentgenograms of the chest.

Of the 211 patients, 184 (87.2 per cent) were Negroes and 27 (12.8 per cent) were Caucasian.

‡ The graduate Hospital of the University of Pennsylvania, the Philadelphia General Hospital, the Woman's Medical College Hospital, the Veterans Administration Hospital in Philadelphia and the Valley Forge Army Hospital, Phoenixville, Pennsylvania.

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TABLE I

MORTALITY RATES OBSERVED IN VARIOUS STUDIES OF PATIENTS WITH SARCOIDOSIS; RELATIONSHIP TO RACE AND TO OCCURRENCE OF CUTANEOUS AND OCULAR INVOLVEMENT

Location	Number of Patients	Negroes (%)	Cutaneous Lesions (%)	Ocular Lesions (%)	Deaths		References
					No.	%	
London	200	...	16.5	25	11	5.5	James [9]
Boston	52	15.4	63.4	27	3	5.8	Longcope and Freiman [10]
London	102	...	16.7	16.7	7	6.9	Scadding [11]
Rochester, Minnesota	194	4.6	...	...	17	8.8	Carr and Gage [12]
Holland	150	...	6.7	18	14	9	Ten Have [13]
Philadelphia	211	87.2	26.5	17.6	20	9.5	Present report
Paris	41	...	9.8	31.7	6	14.6	Turiaf and Brun [14]
U. S. A. (veterans)	540	...	...	...	84	15	Cummings, Dunner and Williams [15]
Philadelphia	97	77.7	46.4	...	15	15.5	Sones, Israel and Stein [16]
Oslo	90	...	44.4	25.5	16	17.7	Nitter [4]
New York	35	85.7	40	20	7	20	Reisner [17]
Copenhagen	191	...	100	...	44	23	Gilg [18]
New York	52	79.0	25	29	13	25	Riley [3]
Baltimore	32	81.1	22.5	64.4	9	28.1	Longcope and Freiman [10]

Most patients were between twenty and forty years of age. Two-thirds were females (Table II). The mean age of white patients was 30.5 years, of Negro patients 27.3 years. More than 80 per cent of the Negro patients were born in the southeastern United States; however, a similar geographic origin was noted in patients with tuberculosis examined at the Henry Phipps Institute in Philadelphia [5]. A history of household or other close contact with tuberculosis was elicited in 16.7 per cent.

Ninety-three patients received treatment with corticosteroid hormones during the course of their disease. Eighty-four were given adrenal steroid hormones (cortisone, prednisone, prednisolone and triamcinolone). In six patients ACTH and adrenal steroids were administered; three patients were given ACTH alone. The treated and untreated groups were analyzed separately and the results revealed little difference (Table III). The more favorable results in the untreated group clearly reflected the practice of withholding treatment in asymptomatic patients. More precise determination of the effects of steroid therapy would require paired controls and a number of patients larger than have as yet been collected in one clinic. It is our opinion that although corticosteroids definitely modify the clinical symptoms and pathologic changes, the ultimate outcome of the disease is not as a rule significantly altered by use of these agents. Similar observations have been made in asthma [6]. Hence it appears justifiable to group together treated and untreated cases in studying the course of sarcoidosis in our patients.

SURVIVAL RATES

Despite the difficulty of tracing patients with a relatively mild or benign illness, we were able to examine or secure information concerning a high percentage of patients. (Table IV.) Twenty patients are known to have died during the period of observation. It is noteworthy that all were Negroes. No deaths occurred in the first year after diagnosis but there were four in the

TABLE II

AGE, RACE AND SEX OF 211 PATIENTS WITH SARCOIDOSIS (PHILADELPHIA 1938 TO 1958)

Age	Caucasian		Negro	
	Male	Female	Male	Female
Under 10	0	0	2	1
10–19	2	0	6	11
20–29	6	5	36	71
30–39	6	4	11	33
40–49	0	2	2	8
50–59	0	1	0	3
60–69	0	1	0	0
Total	14	13	57	127

TABLE III
STATUS OF 211 PATIENTS WITH SARCOIDOSIS AT END OF OBSERVATION PERIOD; COMPARISON OF 93 PATIENTS TREATED WITH CORTICOSTEROIDS AND 118 UNTREATED PATIENTS (PHILADELPHIA 1938 TO 1958)

Treatment	Cleared		Improved		Unchanged		Worsened		Died	
	No.	%	No.	%	No.	%	No.	%	No.	%
Corticosteroids	23	24.7	33	35.5	3	3.2	23	24.7	11	11.8
No steroids	50	42.4	34	28.8	14	11.8	11	9.3	9	7.6

second year. One death occurred in the third year, three in the fourth year, and seven in the fifth year. Single deaths occurred in the eighth, tenth, thirteenth, nineteenth and twentieth years after diagnosis.

Survival rates were calculated employing the life table method in which it was assumed that the mortality among those lost to observation was the same as among those followed up. This assumption may exaggerate slightly the mortality rates, since it is our belief that the patients lost to observation were often those who recovered promptly and saw no need for further medical care.

It is noteworthy that the survival rate of 88.8 per cent five years after diagnosis is considerably lower than the life expectancy (98.0 per cent) calculated for a normal population of this age, sex and race. Similarly, the survival rate of 84.8 per cent ten years after diagnosis is further reduced from the expectancy of 95.2 per cent survival calculated for a normal population. Thus sarcoidosis lowers life expectancy to a significant degree.

TABLE IV
SURVIVAL RATE OF 211 PATIENTS WITH SARCOIDOSIS (PHILADELPHIA 1938 TO 1958)

Years	Number Alive	Number Dead*	Number Lost to Follow-up	Estimated Survivals	Survival Rate† (%)	Survival Rate for Normal Population‡ (%)
0	211	...	...	...	...	...
1	197	0	14	211	100.0	99.6
2	171	4	22	206.2	97.7	...
3	151	1	19	204.8	97.1	98.8
4	123	3	25	199.9	94.7	...
5	104	7	12	187.3	88.8	98.0
6	85	0	19	187.3	88.8	...
7	70	0	15	187.3	88.8	...
8	56	1	13	184.0	87.2	...
9	42	0	14	184.0	87.2	...
10	35	1	6	178.9	84.8	95.2

* Three deaths occurred after the tenth year.
† Assumption is made that death rate of those observed and those lost to observation is the same.
‡ Estimated for a population of similar age, sex and race.

Cause of Death. Necropsy reports were available in eleven of the twenty patients known to have died. (Table v.) Designation of the probable cause of death in the other instances has been made on the basis of all available clinical and bacteriologic data. The diagnosis of tuberculosis has not been accepted in the absence of bacteriologic evidence. Three deaths were unquestionably due to tuberculosis, one to intercurrent infection with anthrax, and the remaining sixteen were attributable to sarcoidosis. One patient died of myocardial sarcoidosis, five of cor pulmonale, nine of respiratory failure, and one from pulmonary hemorrhage.

STATUS AT END OF THE OBSERVATION PERIOD

The interval of observation after diagnosis of the 211 patients ranged from three months to twenty years, with a mean of 5.9 years.

Complete clearing was observed in 73 (34.6 per cent) of the group and an additional 67

TABLE V
OBSERVATIONS ON 211 PATIENTS WITH SARCOIDOSIS; CAUSE OF DEATH IN 20 PATIENTS (PHILADELPHIA 1938 TO 1958)

Cause of Death	Necropsy	No Necropsy
Respiratory failure	3	6
Cor pulmonale	4	1
Myocardial sarcoidosis	1	...
Pulmonary hemorrhage	1	...
Pulmonary tuberculosis	1	2
Anthrax	1	...
Total	11	9

(31.8 per cent) were classified as improved. (Table VI.) The disease remained unchanged in seventeen instances and in thirty-four patients (16.1 per cent) grew worse. As already noted, 20 patients (9.5 per cent) died.

We have defined clearing as complete subsidence of symptoms, signs and radiologic evidence of the disease. The time of clearing shown by the roentgenographic changes in the seventy-three patients in this category is of interest; in twenty-five of these patients (11.9 per cent of the original number) clearing occurred in the first year after diagnosis; in twenty-eight patients (13.2 per cent) in the second year of observation, and in another eleven (5.2 per cent) in the third year. It should be noted that nine patients (4.3 per cent) showed complete clearing more than three years after the initial diagnosis.

The mean time of clearing in fifty untreated patients was 22.2 months while in twenty-three patients treated with adrenal steroids the mean time of clearing was 20.6 months. The difference is not statistically significant, but it should be noted again that the treated and untreated groups were dissimilar and not comparable.

The number of patients classified as improved was sixty-seven, which represents 31.8 per cent of the entire group. Half the patients were treated with corticosteroids. Ten required adrenal steroid drugs continuously or intermittently in order to maintain improvement.

Thirty-four patients (16.1 per cent) were classified as having progressive disease at the end of the observation period. Twenty-three patients had been given corticosteroid drugs and it is noteworthy that their use in these instances had not prevented progression.

Seventeen patients (8.1 per cent) have been classified as unchanged. In many instances the failure to note change was due to early loss from observation, but eight patients were observed for more than three years without apparent change in roentgenograms of the chest.

TABLE VI
OBSERVATIONS ON 211 PATIENTS WITH SARCOIDOSIS
STATUS AT END OF OBSERVATION PERIOD
(PHILADELPHIA 1938 TO 1958)

Condition at End of Observation	Patients		Mean Duration of Observation (yr.)
	No.	%	
Cleared	73	34.6	5.88
Improved	67	31.8	6.25
Unchanged	17	8.1	4.07
Worse	34	16.1	6.28
Died	20	9.5	5.96

INFREQUENCY OF RECURRENCE IN SARCOIDOSIS

Recurrence after complete clearing has occurred but is rare, having been observed in only two instances. In both patients pregnancy may have been a factor. The first was a twenty-two year old Negro woman in whom bilateral hilar adenopathy was discovered in May 1951. A biopsy of a cervical lymph node revealed sarcoid tubercles. Seven months later she was completely well and the lung lesions had disappeared. In August 1955, during her second pregnancy, symptoms of fatigue, weight loss, cough, dyspnea and expectoration developed, and roentgenograms of the chest showed extensive bilateral densities. Since results of the tuberculin test were still negative, the disease was considered to be sarcoidosis. Examination in February 1959 showed clinical improvement although roentgenograms of the chest showed changes were still present.

The second patient was a twenty-three year old Negro woman in whom roentgenographic examination of the chest in April 1953 revealed bilateral hilar adenopathy and extensive miliary densities. Results of the tuberculin test were negative and biopsy of a cervical lymph node showed typical sarcoid granulomas. Because of progressive symptoms and radiologic changes, a short course of cortisone was given (2.35 gm. during a period of one month). Gradual improvement resulted, with complete symptomatic and roentgenographic clearing by December 1955. In April 1957, three months following the termination of a normal pregnancy, miliary densities were again noted, which persisted to August 1957. Gradually, improvement ensued and by April 1958 complete clearing was observed.

Although histologic evidence to support the diagnosis of sarcoidosis was not obtained during the relapses, it appears probable on clinical and radiologic grounds that these patients had recurrence of sarcoidosis.

SARCOIDOSIS AND TUBERCULOSIS

In addition to three fatalities, in four other patients tuberculosis developed, with acceptable

TABLE VII
RELATION OF INITIAL ROENTGENOGRAM OF THE CHEST TO COURSE OF DISEASE IN 211 PATIENTS WITH SARCOIDOSIS
(PHILADELPHIA 1938 TO 1958)

Initial Roentgenogram	Number of Patients	Cleared or Improved		Unchanged or Worse		Deaths	
		No.	%	No.	%	No.	%
Negative	12	9	75.0	3	25.0	0	...
Mediastinal adenopathy	71	46	64.8	21	29.6	4	5.6
Infiltration alone	56	34	60.7	15	26.8	7	12.5
Adenopathy and infiltration	72	51	70.8	12	16.7	9	12.5

bacteriologic proof of the diagnosis. In five additional patients a diagnosis of tuberculosis was considered because of positive smears with negative cultures or guinea pig inoculations or because of a single positive report among many negative sputum or gastric cultures. We have not regarded such patients as having tuberculosis.

In five of the seven patients in whom tuberculosis developed results of the tuberculin test were negative when the diagnosis of sarcoidosis was first made, and the tuberculosis which developed from two to five years subsequently is believed to have been the result of later infection. Two patients were reactors to second strength P.P.D. when first diagnosed as sarcoidosis. One patient had clinical features characteristic of sarcoidosis, and tuberculosis developed nineteen years after his initial observation. In the second patient pleural effusion and tuberculin conversion was observed two months after he entered our clinic and it seems highly probable that this was an error in diagnosis, i.e., he actually had atypical tuberculosis rather than sarcoidosis.

Although the greater frequency of tuberculosis as a sequel to sarcoidosis has been considered analogous to the greater susceptibility of silicotic patients to tuberculosis, only four of the seven patients in whom tuberculosis supervened had extensive pulmonary damage. In three patients tuberculosis developed more than a year after the sarcoid lesions had completely cleared.

In only one patient in whom tuberculosis developed had corticosteroid therapy been given. This was a patient with extensive and severe pulmonary involvement due to sarcoidosis who was treated with adrenal steroids for twenty-one months, with considerable improvement. Three months after the steroid therapy was discontinued there was acute onset of fever and cough which proved to be due to tuberculosis.

ERYTHEMA NODOSUM AND SARCOIDOSIS

Erythema nodosum was recognized in only six (2.9 per cent) of our patients, two Caucasian females and four Negro females. This is a frequency much lower than that observed in Northern Europe, where erythema nodosum has been reported in a high proportion of Swedish patients [7] and in 31 per cent of English patients with sarcoidosis [8].

RELATIONSHIP OF ROENTGENOGRAPHIC CHANGES OF THE CHEST TO PROGNOSIS

The initial roentgenogram of the chest revealed no abnormalities in twelve patients. (Table VII.) In seventy-one patients mediastinal adenopathy alone was observed, and parenchymal infiltration alone was noted in fifty-six patients. In seventy-two patients both mediastinal adenopathy and parenchymal infiltration were present.

European investigators have applied the term "hilar lymphoma syndrome" to patients with hilar adenopathy as a dominant feature, and have emphasized the excellent prognosis in these cases. Among seventy-one patients in this study whose initial roentgenographic examination showed mediastinal adenopathy alone, only 21 (29.6 per cent) cleared completely. Clearing was complete within the first year in five patients, in the second year in nine patients, and in the third year in two patients. Five patients required more than three years to clear. The difference in behavior between European patients and those in the present study is a striking one.

It is noteworthy that in twenty-five patients who initially had mediastinal adenopathy, parenchymal infiltration subsequently developed. The reverse did not occur, that is, in no case was mediastinal adenopathy observed to occur after pulmonary disease. The sequence of events in sarcoidosis is in striking contrast to the pattern of tuberculous infection.

The findings on the initial roentgenogram of the chest showed little correlation with the outcome at the end of the observation period. (Table VII.) Whether there was adenopathy, infiltration, or both, two-thirds of the patients improved. Patients with mediastinal adenopathy

alone had a mortality rate of 5.6 per cent as compared to a mortality rate of 12.5 per cent for patients with parenchymal infiltration, but these differences are not significant.

FACTORS AFFECTING THE PROGNOSIS

As was noted, clearing or improvement occurred in 140 patients and fifty-four patients either became worse or they died. An attempt has been made to determine the influence on outcome of various factors. (Table VIII.) It will be noted that the mean age and the sex distribution of patients who improved and those whose condition became worse were essentially similar. A significant racial difference was demonstrable. The percentage of Caucasian patients among those who improved was 14.3 per cent, as compared to 7.4 per cent among patients whose condition became worse. Among the fifty Negro patients who had progressive disease, there were twenty deaths, while among Caucasians there were four who showed progression, with no deaths. The difference in mortality is not a significant one.

As will be shown, the presence of cutaneous lesions appears to be a significant prognostic feature. It is noteworthy, therefore, that only three or 11.1 per cent of Caucasian patients had cutaneous sarcoids, while fifty-three or 25.1 per cent of Negro patients had dermal involvement. The less favorable outcome in Negroes may be related more to the greater frequency of skin lesions in this group than to any other racial difference.

Among the patients who improved, 25 per cent showed involvement of three or more systems, while among patients with progressive disease 42.6 per cent had similarly disseminated involvement. Patients who had cutaneous and osseous involvement were significantly more often in the group with progressive disease, while no significant differences were demonstrable in relation to involvement of the liver, spleen, eyes or chest.

Analysis by subgroups indicates that the differences observed according to race and according to the number of organs or systems involved are actually related to the occurrence of cutaneous involvement. The difference in prognosis between patients with and without osseous lesions appears to be the result of the difference in frequency of associated skin lesions. The difference in prognosis between patients with cutaneous sarcoids and those without cutaneous disease is the only one consistently significant in homogeneous subgroups.

TABLE VIII
A COMPARISON OF FACTORS WHICH MIGHT AFFECT PROGNOSIS IN 194* PATIENTS WITH SARCOIDOSIS (PHILADELPHIA 1938 TO 1958)

Factor	Condition of Patient		Difference Significant at 0.05 Level
	Improved (140, mean age 27.3 yr.) (%)	Worsened (54, mean age 28.8 yr.) (%)	
Sex (male)	36.4	33.4	No
Race: Caucasian	14.3	7.4	Yes
Negro	85.7	92.6	Yes
Three or more systems involved	25.0	75.0	Yes
Chest involvement alone	38.6	29.6	No
Liver or spleen	24.3	31.5	No
Skin	20.7	44.4	Yes
Eyes	16.4	20.4	No
Bones	12.1	24.1	Yes

* Seventeen patients whose disease was "unchanged" at end of observation period are omitted from this analysis.

Thus, if all patients with involvement of the skin are analyzed separately, the differences in prognosis between Caucasians and Negroes, between patients with less than three and those with three or more systems involved, and between those with and without osseous involvement, disappear. Similarly, if patients without involvement of the skin are analyzed, the differences in race and system involvement also disappear. The difference in prognosis between patients with and without cutaneous disease remains significant regardless of race and other system involvement.

COMMENTS

Sarcoidosis as observed in Philadelphia over the past two decades is neither as benign as reports from Boston [*1,10*] and London [*9,11*] indicate nor as malignant as studies from Oslo [*4*], Copenhagen [*18*] and New York [*3,17*] have shown. In Table I are listed, in order of increasing mortality rates, some of the principal reports on the course of sarcoidosis. Wherever specified, data on the race of patients and the

frequency of cutaneous and ocular involvement have been included. The extraordinary variation in death rates, ranging from zero to 28 per cent, is undoubtedly the result of many factors. Before considering the possibility of geographical influences, other causes for the varied observations on the seriousness of sarcoidosis deserve comment.

The length of observation influences the frequency with which complete clearing or a fatal outcome will become manifest. Although the lesions may disappear within a few months in some patients, in other instances clearing was noted only after four or five years of observation. No deaths occurred among patients in the present series during the first year after diagnosis, and it is evident that a falsely optimistic view of the mortality from the disease will be obtained if patients are observed for only short periods.

In an earlier analysis [*16*] of the records of ninety-nine patients with sarcoidosis treated in Philadelphia hospitals and clinics from 1938 to 1947, the fatality rate was 15.5 per cent. This rate was high because of the selected population studied; the majority were ill enough to be hospitalized. The high mortality found in New York [*3*] and Baltimore [*10*] presumably also reflects the more serious prognosis of patients encountered in hospital studies. In the present study, clinic and office patients were in the majority; many had scant symptoms and received study only because of abnormalities detected by survey roentgenograms of the chest. The present data likewise indicate that the prognosis varies according to the organs involved, so that differences in outcome may be anticipated in studies in which patients with cutaneous or ocular or pulmonary manifestations predominate.

To some extent, also, the results are influenced by the diagnostic criteria employed. In a number of studies the diagnosis of sarcoidosis has in some instances been made on clinical and roentgenologic findings alone, which introduces a considerable error. In other series the diagnosis has often been based on the Kveim reaction, the clinical reliability of which the present authors believe to be open to question [*19*]. In some studies it seems evident that instances of isolated sarcoid reaction have been erroneously included as cases of sarcoidosis. In the present report, consideration has been restricted to cases in which a clinical diagnosis of systemic sarcoidosis has been supported by one or more biopsies. On the whole, however, the error introduced by the differences just enumerated is a minor one, consisting at most of the inclusion in some series of a few cases of atypical tuberculosis and of carcinoma. The vast majority of patients included in European and American studies fulfill the criteria for diagnosis we have adopted.

To return to the question of geographic differences, independent of the variables which we have just considered, it seems safe to conclude that the observations in this study resemble those reported by Nitter in Norway [*4*], Carr and Gage in the United States [*12*], Turiaf and Brun in France [*14*], Ten Have in Holland [*13*], and Scadding in England [*11*]. The close correspondence of these studies as far as mode as well as frequency of death are concerned supports the belief that sarcoidosis is a disease entity essentially identical in Europe and North America.

There are, however, two notable differences between sarcoidosis in Northern Europe and in the United States. One is the great frequency of erythema nodosum as an early manifestation of sarcoidosis in Europe; the other is the difference in behavior of patients with hilar lymphadenopathy. The lower frequency with which erythema nodosum is observed in American patients with sarcoidosis may be the result of several factors: (1) erythema nodosum may be more difficult to detect in Negroes; (2) exposure to the inciting agent of sarcoidosis may be less explosive or intense in the United States; and (3) nutritional or other environmental factors may account for host differences in reactivity. It should be remembered that erythema nodosum is an equally uncommon accompaniment of primary tuberculous infection in the United States [*20*].

English and Swedish reports agree that the "hilar lymphoma syndrome" is almost invariably benign. Löfgren [*7*] in Sweden noted that 72.6 per cent of sixty-two patients with bilateral hilar adenopathy discovered on routine examination regressed within two years. Smellie and Hoyle [*2*] in England reported clearing in 68.2 per cent of sixty-six patients with hilar lymphadenopathy and remarked that in most instances clearing was complete within one year. Complete clearing was noted in only 29.6 per cent of our seventy-one patients with hilar adenopathy, and disappearance of the lymph node masses often required many years. The outcome in our patients with hilar adenopathy did not differ significantly from that of patients

with parenchymal involvement. Progressive disease and death occurred almost as often among those whose initial roentgenogram of the chest showed only hilar adenopathy as among those who had pulmonary infiltration. No explanation is evident for the difference in course of this type of sarcoidosis in Northern Europe and in Philadelphia.

The relatively low incidence of tuberculosis (3.3 per cent) encountered in this study is worthy of special note. In seven patients tuberculosis developed, which in three instances was fatal; in only one patient was adrenal steroid therapy a possible factor. Since nearly half our patients were treated with corticosteroids, it is remarkable that tuberculosis supervened in only one. The high incidence of tuberculosis noted in other studies [*3,17,21*] may well be the result of the greater possibility of tuberculosis superinfection in previous decades and in other countries, as well as a tendency in the past to make the diagnosis of tuberculosis on the basis of radiologic or inadequate bacteriologic evidence.

Although the development of tuberculosis in 3.3 per cent of the patients in the present study is low in contrast to the 25 per cent noted at Bellevue Hospital [*3*] and other older studies [*12*], the incidence observed in our patients is clearly in excess of that anticipated in a normal population at the present time. The slightly increased hazard of tuberculosis in patients with sarcoidosis may be the result of the following factors: (1) the pulmonary fibrosis which may result from sarcoidosis predisposes to tuberculosis; (2) sarcoidosis and tuberculosis are different diseases but resemble each other so closely that they cannot be distinguished with accuracy; (3) sarcoidosis is characterized by altered serum globulins and immunologic abnormalities [*22*] which could result in diminished resistance to infection; and [*4*] social and economic factors responsible for sarcoidosis may include some which enhance the likelihood of tuberculosis. The observations in the present study are not consistent with the first hypothesis. It is undoubtedly true that sarcoidosis and tuberculosis are at times mistaken for each other, but our experience indicates that careful study can differentiate the two in most instances. The third hypothesis seems unlikely since tuberculosis developed in many of our patients at a time when the sarcoidosis had cleared and the immunologic changes were subsiding. Although we know of no evidence which directly supports the fourth hypothesis it seems to us to be the most reasonable explanation.

The problem of confusion with fungal disease deserves discussion. Two patients who were suspected as having sarcoidosis have not been included in this study because diagnosis proved that they had histoplasmosis. Another patient was diagnosed as having sarcoidosis in 1951; in 1953 cryptococcosis developed [*23*], apparently representing a superinfection. In no other instance during the period of observation did fungal disease become manifest.

Calculation of mortality and survival rates by use of the life table method has been employed to our knowledge in only one other study of sarcoidosis, that of Carr and Gage [*12*]. Among patients with sarcoidosis studied at the Mayo Clinic, the five-year and ten-year survival rates were 92.9 per cent and 80.0 per cent respectively as compared to our five-year survival rate of 88.8 per cent and ten-year survival rate of 84.8 per cent. Their material, it should be noted, differs significantly from that of other investigators; the average age was considerably older (41.2 years) resulting in a rather high death rate from neoplasm and other incidental diseases. The frequency of neoplasm suggests that some of the patients included by these investigators did not actually have sarcoidosis; they were perhaps so diagnosed on the basis of histologic findings of sarcoid tubercles in lymph nodes draining an area which contained a malignant neoplasm, an occurrence to which several investigators [*24,25*] have called attention. The patients observed in Philadelphia were much younger, and no deaths occurred from degenerative disease. The slightly lower five-year survival rate in Philadelphia is, therefore, the more impressive. The slightly higher mortality from sarcoidosis in our patients may be the result of racial and social differences.

Clinical and roentgenologic data have been analyzed in an effort to determine the features which would permit more accurate estimation of the prognosis. European investigators have emphasized the difference in outlook between patients with pulmonary involvement and those having only hilar adenopathy [*26*]. In our experience, this difference has been slight and of no practical value in estimating prognosis. Progressive disease was significantly more frequent in Negro patients, in patients with involvement of three or more organs or systems, and especially in patients with cutaneous sar-

coidosis. However, analysis by homogeneous subgroups indicates that the only real difference in prognosis was that related to involvement of the skin.

The ominous prognostic significance of cutaneous lesions in sarcoidosis has not been previously emphasized. It may be seen in Table 1 that among Gilg's patients in Denmark [*18*], all of whom had sarcoids of the skin, the fatality rate was very high. James [*9*] noted four deaths among thirty-three patients with cutaneous sarcoidosis and eleven deaths among 167 patients without cutaneous lesions, a difference of borderline statistical significance. The largest proportion of subjects with cutaneous lesions to be found in American studies is among the patients from the Massachusetts General Hospital, and in this series the mortality was low [*10*]. It is evident that additional observation over prolonged intervals will be needed for confirmation of our finding of the poor prognosis among patients with cutaneous sarcoidosis.

In the introduction of this report the hope was expressed that observations on the natural history of sarcoidosis might afford clues as to its nature and pathogenesis. From this aspect our findings are disappointingly meager. Two observations, however, deserve emphasis. The rarity with which fungal, tuberculous or other bacterial sequellae followed the use of corticosteroid treatment of sarcoidosis argues against an infectious causation of this disease. The infrequency with which recurrence was encountered indicates that although sarcoidosis may represent a hypersensitivity reaction [*27*], it does not share the relapsing pattern so characteristic of the "collagen" diseases.

SUMMARY

1. Observations are presented on the course of sarcoidosis in 211 patients, 184 Negroes and twenty-seven Caucasians, observed in Philadelphia for as long as twenty years, with a mean interval of 5.9 years.

2. Survival rates, calculated by the life table method, were 88.8 per cent after 5 years of observation and 84.8 per cent after ten years of observation. Comparison with the life expectancy of normal persons similar in age, sex and race indicates a considerable diminution of survival as the result of sarcoidosis.

3. Complete recovery was observed in 34.6 per cent and improvement in 31.8 per cent, while in 16.1 per cent the condition became worse. The mortality rate was 9.5 per cent, with sixteen deaths due to sarcoidosis, three to tuberculosis, and one to incidental disease. In four other patients tuberculosis developed, one of whom was among the ninety-three patients treated with corticosteroids. In only two instances was recurrence of sarcoidosis observed.

4. The incidence of erythema nodosum was much less frequent than reported in English and Scandinavian studies, and clearing of mediastinal adenopathy occurred less often and less rapidly than in these two countries. With these exceptions, the course of sarcoidosis appears to be similar in Philadelphia and in Northern Europe.

5. Although the prognosis appears to be less favorable in Negro patients and in those patients with many systems involved, analysis indicates that cutaneous involvement is the feature most closely associated with progressive and fatal sarcoidosis.

Acknowledgment: We are indebted to Drs. E. R. Long, J. L. Wilson, the late J. D. Aronson, H. W. Hetherington and other members of the medical, nursing and administrative staffs of the Henry Phipps Institute for the cooperation which made possible the prolonged observation of patients in the Sarcoid Clinic. The assistance of Drs. D. D. Harrell, A. M. Sheffer, E. Polish, K. G. Alderfer and J. Seymour, R.N. of the Graduate Hospital, University of Pennsylvania, is also acknowledged. We are further indebted to Drs. K. R. Boucot, M. J. Sokoloff and S. C. Stein of the Division of Tuberculosis, Philadelphia Department of Health for referral of patients from survey units and city chest clinics.

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An "Epidemic" of Chemical Meningitis*

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MENINGITIS following the administration of a spinal anesthetic may be the consequence of a number of factors: trauma from the procedure itself, aggravation of an underlying disease of the central nervous system, contamination with viable bacteria, inflammatory response to the anesthetic agent or medication, or an inflammatory response to a foreign irritant (chemical) which has contaminated the equipment used during the anesthetic procedure.

During an eleven-month period we observed five patients in whom an acute purulent "aseptic" meningitis developed shortly following spinal anesthesia which seemed to be a result of the chance use of syringes contaminated with trace amounts of a phenolic disinfectant. Based on these observations and review of previous clinical and experimental studies, the pathogenesis, clinical features and means of prevention of this syndrome are summarized.

CASE REPORTS

The following five women were admitted to Parkland Memorial Hospital for delivery of uncomplicated full-term pregnancies. None demonstrated signs or symptoms of acute or chronic

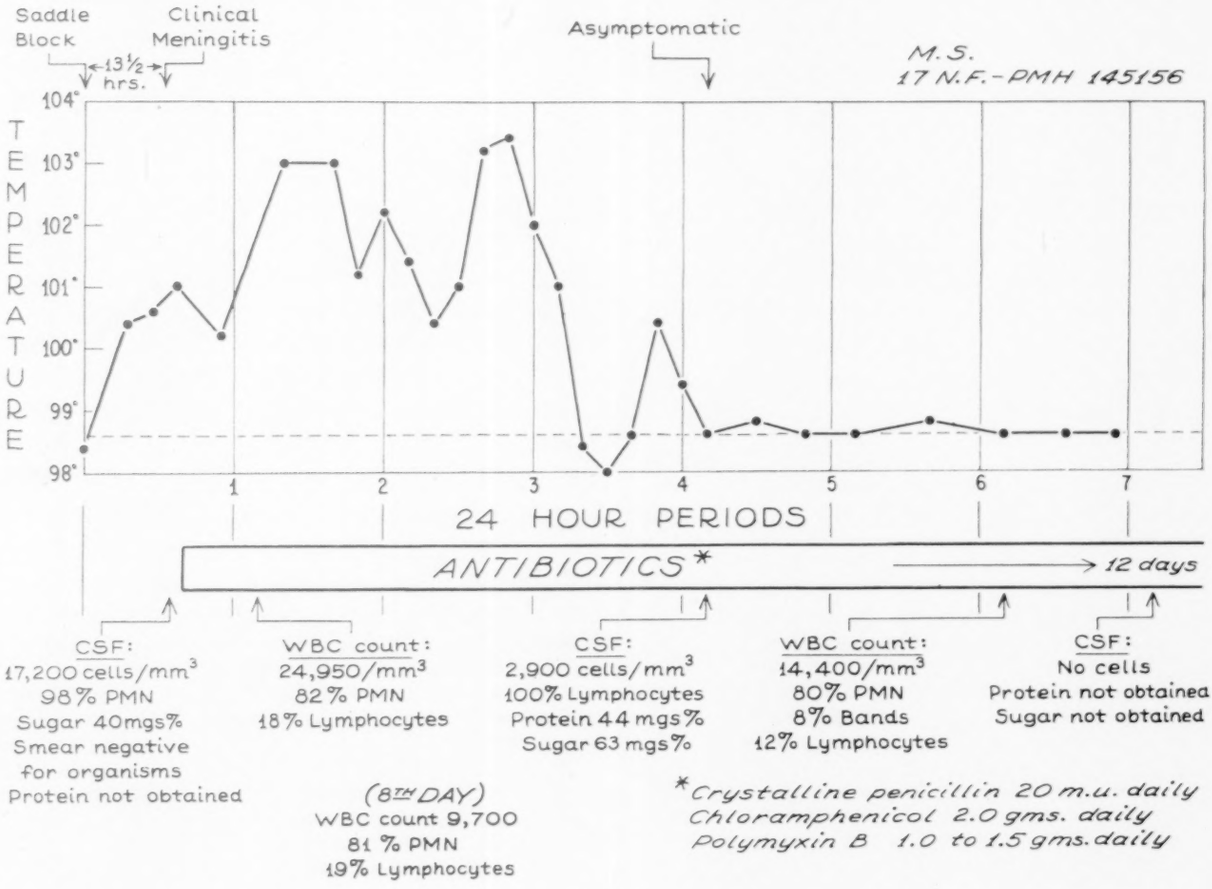


FIG. 1.

* From the Department of Internal Medicine, The University of Texas Southwestern Medical School, Dallas, Texas.

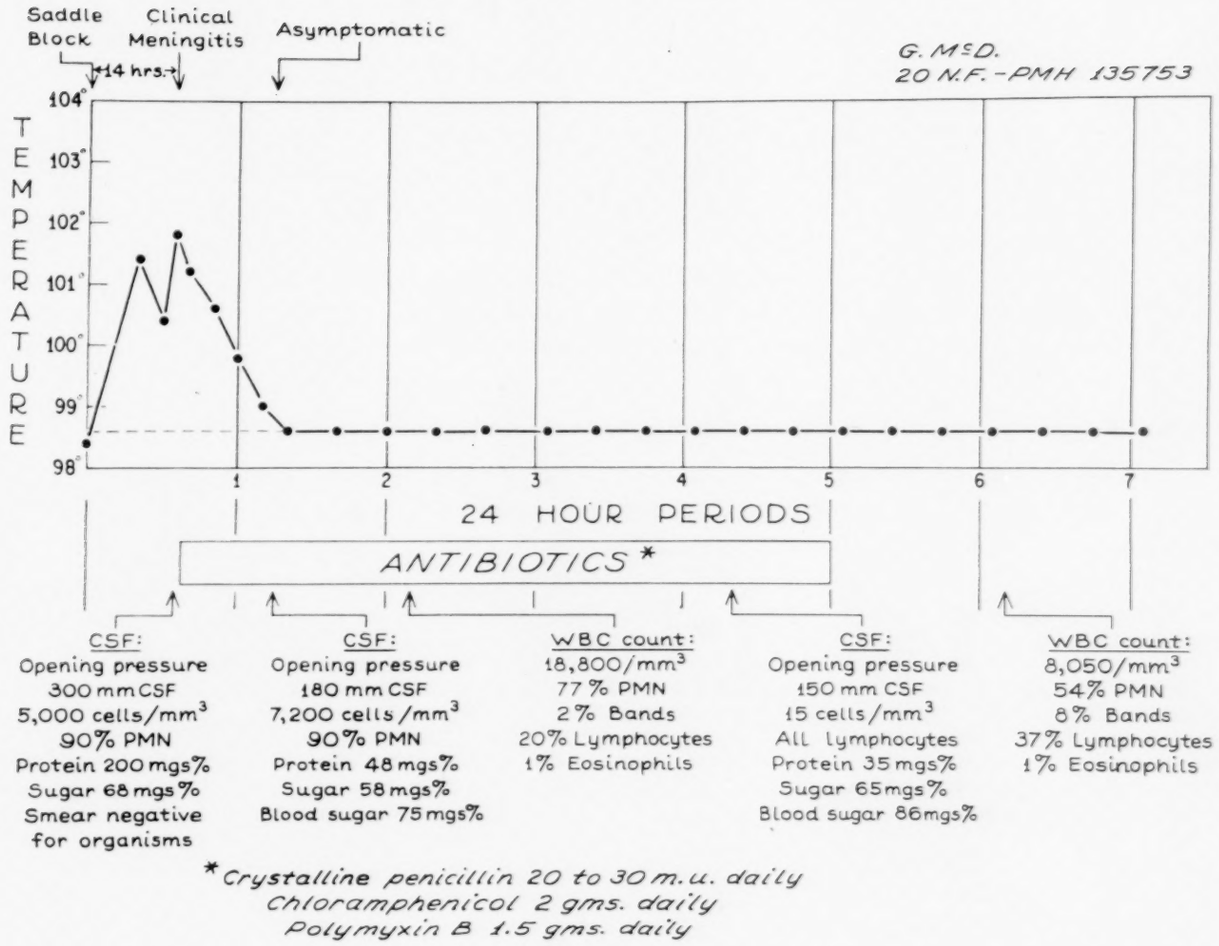


FIG. 2.

disease prior to delivery. These cases are sporadic, considering the number of spinal anesthetics administered during this period by the Department of Obstetrics and Gynecology.

CASE I (PMH No. 145156) (Fig. 1). A seventeen year old gravida 2, para 1 housewife was admitted in September 1957 in active labor. A low spinal anesthetic (saddle block) was administered using 4 mg. tetracaine hydrochloride. Delivery was uneventful. Four hours after administration of the spinal anesthetic her temperature was 100.4°F. orally. Thirteen and a half hours after administration of the anesthetic her temperature was 101°F. and she complained of moderate headache. She was found to have nuchal rigidity. No residual motor or sensory effects of the anesthetic were detected and there were no neurological deficits. Lumbar puncture was performed and the cerebrospinal fluid (CSF) was purulent, containing 17,200 cells/cu. mm., 98 per cent of which were polymorphonuclear leukocytes. No organisms were demonstrated on stained smears of CSF sediment. Blood and CSF cultures were negative. The CSF sugar was 40 mg. per cent. A peripheral white blood cell count obtained twenty-four hours following the onset of meningitis was 15,600/cu. mm. with a shift to the left. Results of serologic tests for syphilis were negative. On the assumption that this represented meningitis secondary to bacterial contamination, the patient was treated with chloramphenicol, penicillin and polymyxin B. Within four days after onset of meningitis she was afebrile and had clinically recovered. Seven days after onset the CSF contained no cells and she was discharged shortly thereafter. She was examined eleven months after this episode and no neurological abnormalities were detected.

CASE II (PMH No. 135753) (Fig. 2). A twenty year old gravida 4, para 1 housewife with two previous spontaneous abortions was hospitalized in March 1958 in active labor. Saddle block anesthesia, using 4 mg. tetracaine hydrochloride, was accomplished with some difficulty. Delivery was uncomplicated. Eight hours

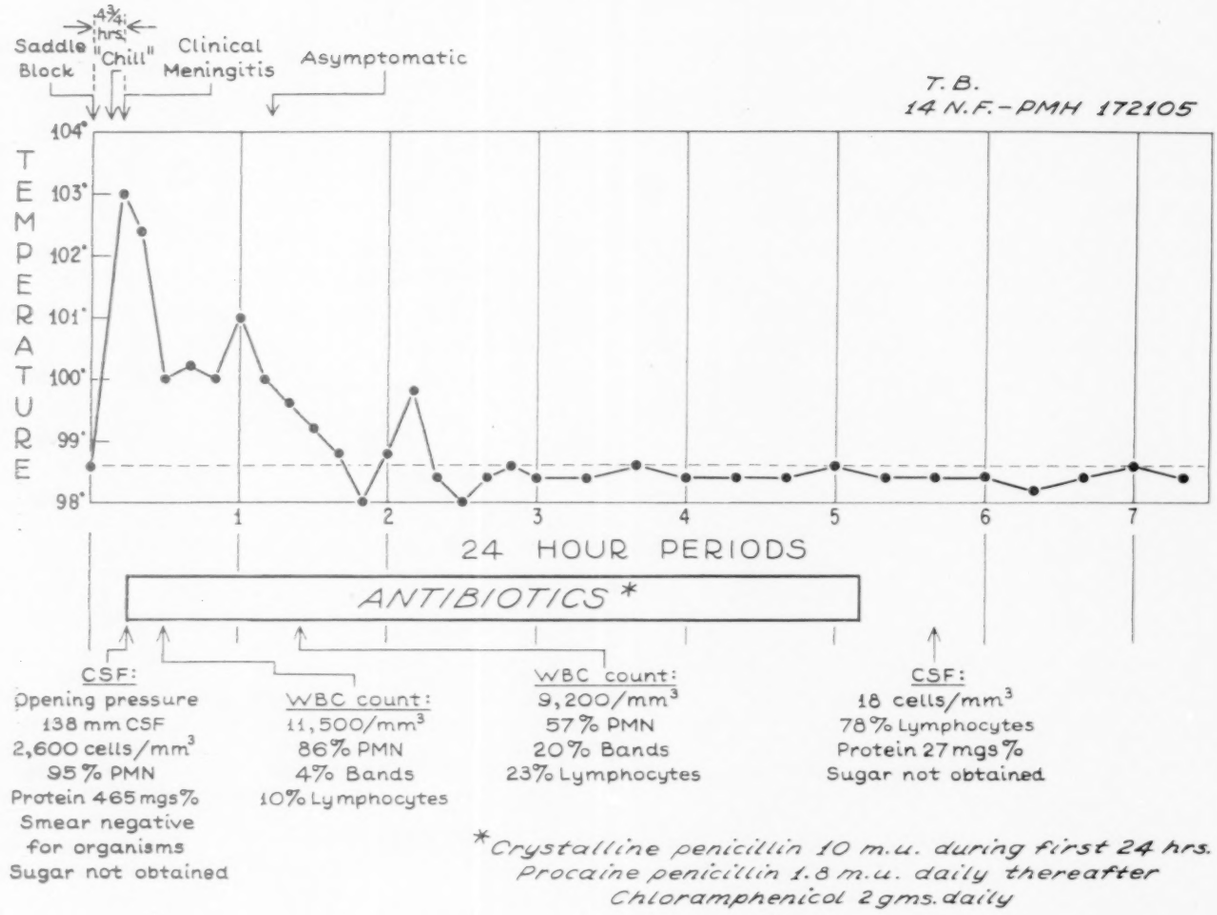


FIG. 3.

after administration of the anesthetic her oral temperature was 101.4°F. rising to 101.8°F. Six hours later severe headache, backache and nuchal rigidity were noted. There were no residual effects of the anesthetic and no neurological changes were detected. On lumbar puncture, the CSF pressure was increased and the CSF contained 5,000 cells/cu. mm. with 90 per cent polymorphonuclear cells, protein 200 mg. per cent and sugar 68 mg. per cent. No organisms were seen on stained smears of the CSF sediment. Blood and CSF cultures were negative. Results of serologic tests for syphilis were negative. Again, bacterial contamination was considered and the patient was treated with penicillin, chloramphenicol and polymyxin B. She had clinically recovered and was afebrile in less than twenty-four hours after onset of the illness. The patient was examined five months after the meningitis and was found to have no neurological abnormalities.

CASE III (PMH No. 172105) (Fig. 3). A fourteen year old girl was admitted in July 1958 in active labor for delivery of her first child. Saddle block anesthesia was performed using 4 mg. tetracaine hydrochloride; an uneventful delivery followed. Approximately four hours after the administration of the anesthetic she had a chill. Within an hour her oral temperature was 103°F. and she complained of severe headache and backache; she was found to have nuchal rigidity. Motor and sensory residuals of the anesthetic were minimal. The CSF contained 2,600 cells/cu. mm. with 95 per cent polymorphonuclear cells, and protein 465 mg. per cent. Organisms were not demonstrated on stained CSF smears and blood, and CSF cultures were negative. Results of serologic tests for syphilis were negative. Shortly after onset of the meningitis the peripheral white blood cell count was 11,500/cu. mm. with a shift to the left. The patient was treated with penicillin and chloramphenicol. Within forty-eight hours she was afebrile and asymptomatic, with no neurological abnormalities.

CASE IV (PMH No. 1575381) (Fig. 4). A seventeen year old primigravida housewife was admitted in active labor in August 1958. Saddle block anesthesia was performed using 4 mg. tetracaine hydrochloride;

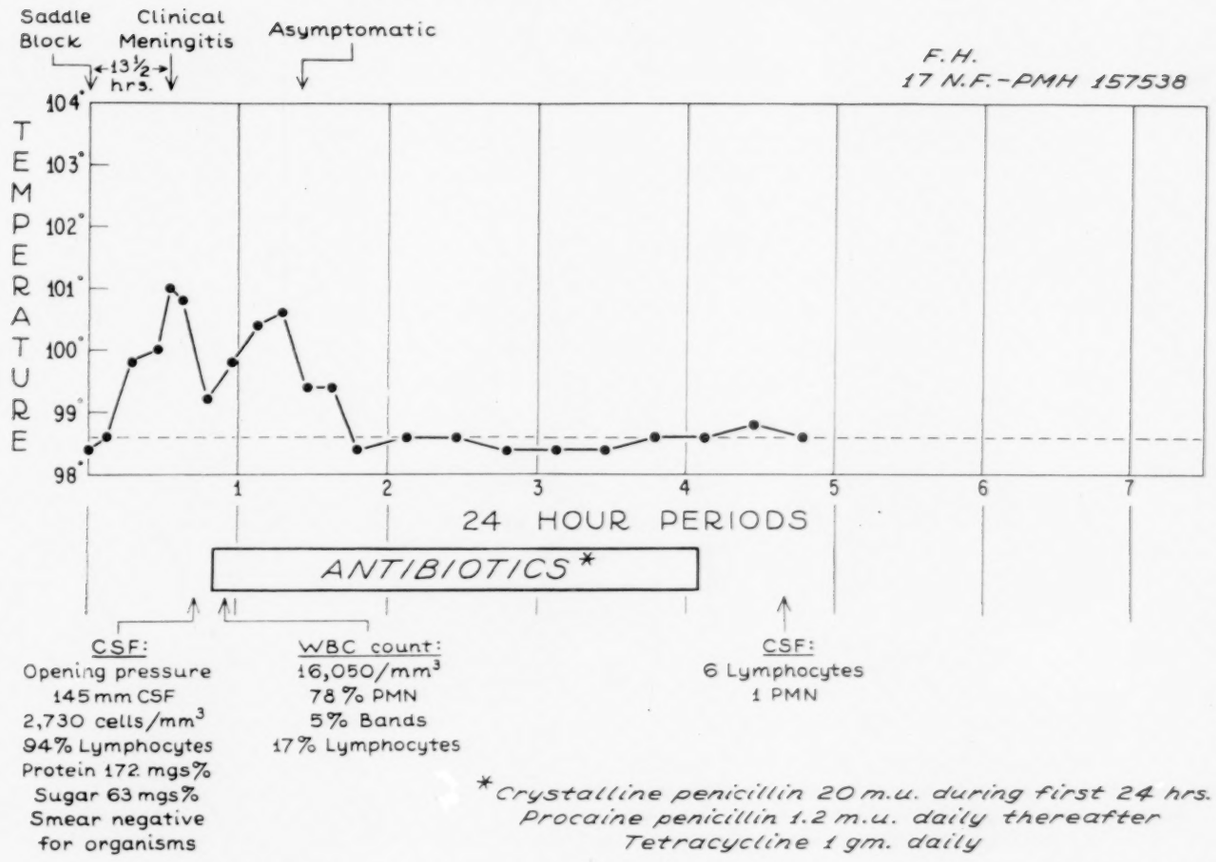


FIG. 4.

an uneventful delivery followed. Thirteen and a half hours after administration of the anesthetic her oral temperature was 101°F. and she complained of headache and backache. There was nuchal rigidity but no other neurological findings. The CSF contained 2,730 cells/cu. mm., 94 per cent of which were reported as "lymphocytes" (this was not checked by the medical staff). The CSF protein was 172 mg. per cent and the sugar was 63 mg. per cent. No organisms were seen on stained smears of the CSF. Blood and CSF cultures were sterile. Results of serologic tests for syphilis were negative. The peripheral white blood cell count shortly after onset of meningitis was 16,050/cu. mm. with a shift to the left. The patient was treated with penicillin and tetracycline. Twenty-four hours after onset of the illness she was asymptomatic and afebrile. There were no neurological changes at the time of discharge.

CASE V (PMH No. 116066) (Fig. 5). The fifth patient differed somewhat from the preceding patients. A thirty-six year old gravida 9, para 7 housewife was hospitalized in August 1958 for Caesarean section and hysterectomy. She had previously undergone Caesarean section for cephalopelvic disproportion and was being treated for iron deficiency anemia attributable to multiple pregnancies and menorrhagia. For some time she had complained of vague headaches for which her attending physician and various consultants had been unable to detect an organic basis. The preoperative peripheral white blood cell count was 6,100/cu. mm.

Two days after admission a Caesarean section and hysterectomy were performed under continuous spinal anesthesia using 3.3 per cent procaine with a catheter introduced intrathecally through the third and fourth lumbar interspace. Surgery was completed three hours after initiation of the spinal anesthetic and the patient was moved to the recovery room. Six and a half hours after the beginning of spinal anesthesia her rectal temperature was 102.2°F. Examination of the chest and legs was described as being within normal limits. Penicillin and tetracycline were given parenterally. Twelve hours after initiation of spinal anesthesia she was returned to her room. At this time she complained of headache. Her oral temperature was recorded as 98.6°F. The neck was "supple." The patient persistently complained of "stabbing" frontal headache and, twenty-seven hours after administration of the anesthetic, her temperature was 101.2°F. orally. Approximately forty-eight hours after administration of the spinal anesthetic her headache

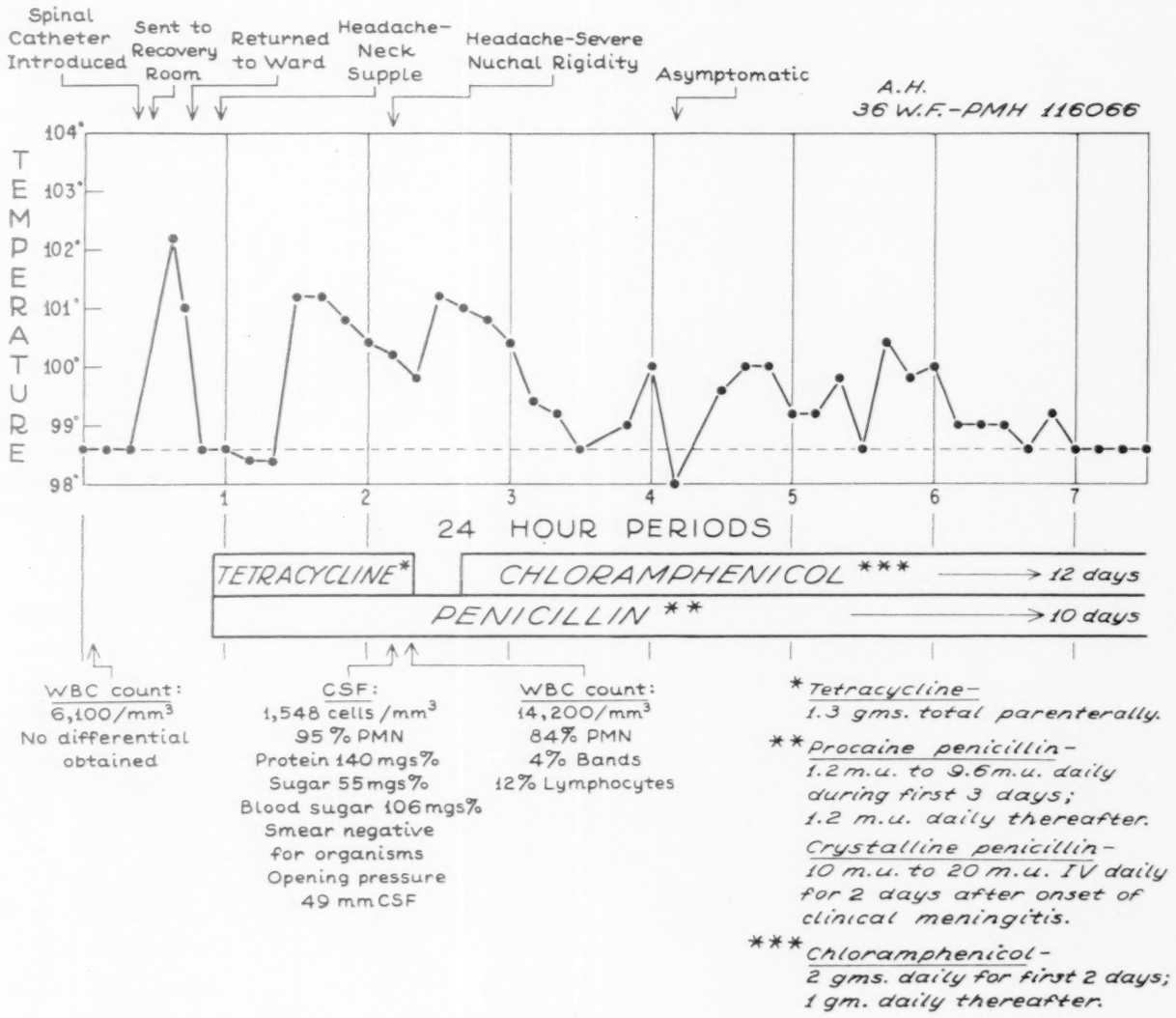


FIG. 5.

worsened and there was definite nuchal stiffness. No other neurological changes were present. A lumbar puncture was performed. The opening pressure was quite low and the CSF contained 1,548 cells/cu. mm., 95 per cent polymorphonuclear cells, with a protein of 140 mg. per cent and sugar of 55 mg. per cent. No organisms were seen on stained smears of the CSF. Blood and CSF cultures were negative. Results of serologic tests for syphilis were negative. Roentgenograms of the skull revealed no abnormalities. The patient was treated with chloramphenicol and penicillin. The peripheral white cell count was 14,200/cu. mm. with a shift to the left.

She was asymptomatic within forty-eight hours following the diagnostic lumbar puncture; however, low grade fever persisted for two additional days. She was discharged with no neurological changes followed with headaches similar to those prior to this illness.

COMMENTS

The similarities in the clinical course of four of these five patients were striking. Within fourteen hours following spinal anesthesia (saddle block) utilizing tetracaine hydrochloride an acute meningitis developed. The finding of purulent spinal fluid with a predominance of polymorphonuclear leukocytes, the presence of fever and of peripheral leukocytosis with a shift to the left strongly suggested bacterial meningitis. The possibility of bacterial contamination as a result of inadequately sterilized anesthetic agent or equipment was considered quite likely. Pseudomonas species or coliform organisms are among the most likely etiologic agents in meningitis following procedures on the central nervous

system, including spinal anesthesia [*1*]. However, in no instance were organisms demonstrated on stained smears of the cerebrospinal fluid sediment and the spinal fluid sugar concentrations were normal. Despite this discrepancy and because of our early failure to appreciate the possibility of purulent sterile meningitis, intensive antibacterial therapy directed against these organisms was initiated in each instance. The failure to culture organisms from either the cerebrospinal fluid or blood suggested the likelihood of a non-bacterial form of postspinal anesthetic meningitis. Another consideration was that the trauma of the procedure had caused bleeding into the intrathecal space. The cerebrospinal fluid was neither bloody nor xanthochromic, and the magnitude of the pleocytosis without frank blood in the cerebrospinal fluid militated against this view. Aggravation of an underlying disease of the central nervous system by spinal anesthesia has been reported, especially in patients with pernicious anemia, multiple sclerosis, tabes dorsalis, general paresis, and metastatic and primary neoplasms of the central nervous system [*2,10*]. None of our patients exhibited the clinical features of these illnesses and follow-up examination have failed to detect such diseases.

Thus, an inflammatory response to a foreign irritant or "chemical meningitis" was suggested. The occurrence of untoward reactions to the intrathecal injection of foreign substances is well documented.

Early in the use of spinal anesthesia, aseptic meningeal reactions manifested principally by a cerebrospinal fluid pleocytosis frequently were reported. Merritt and Fremont-Smith [*3*] reviewed several reports published between 1928 and 1934 in which over one hundred and fifty patients were studied following spinal anesthesia; slight to marked cerebrospinal fluid pleocytosis was found within twelve to twenty-four hours in from 65 per cent to approximately 100 per cent of patients. A predominance of polymorphonuclear cells was demonstrated in some patients, in others the response was lymphocytic. In roughly one-third, there was elevation of the cerebrospinal fluid protein. However, in 1936 Orkin [*4*] summarized the reports of approximately 46,000 administrations of spinal anesthetics and reported an incidence of aseptic meningitis of only 0.26 per cent. Subsequent and more recent studies have not demonstrated a significant incidence of inflammatory response after spinal anesthesia [*5,17*]. Kamsler [*5*] reported no cells in the cerebrospinal fluid in nine of twelve patients given a single injection of an anesthetic agent; 5 cells/cu. mm. was the highest number found in the other three [*5*].

Yet there can be little question that severe neurological complications still may occur following spinal anesthesia [*6–9*]. Two general syndromes have been encountered following spinal anesthesia using procaine or one of its derivatives. One is a destructive neurological process which either may begin promptly, the patient failing to recover fully from the effects of the anesthetic agent or may begin after weeks or months. Such complications are usually progressive and are not associated with fever or overt clinical meningitis. The lesions consist of progressive demyelinization of the spinal roots beginning with the cauda equina, chronic adhesive arachnoiditis, hyperplastic pia with marginal demyelinization of the cord, or, in some cases, progression of the process to a meningoencephalitis and sometimes to internal hydrocephalus. A fatal outcome is not uncommon and residual motor and sensory deficits are frequent [*2,11–13*]. Winkleman [*12*] studied eleven such cases and found that the syringes used for administering the anesthetic agents were cleansed in a "mild detergent solution" with only casual rinsing in tap water before autoclaving. He demonstrated that "injection of a similar mild detergent solution into an animal produced paralysis of the hind legs." Paddison and Alpers [*13*] also reported a fatal case of adhesive arachnoiditis, nerve root and spinal cord degeneration which they attributed to detergent remaining in the spinal anesthesia equipment following washing. Additional experimental support came from the observations of Denson and co-workers [*14,15*]. Using syringes which had been soaked in various detergent solutions, then autoclaved without washing, they injected spinal anesthetic solutions into the subarachnoid spaces of monkeys; most showed varying degrees of arachnoiditis and nerve tissue damage when sacrificed.

The second syndrome encountered following spinal anesthesia is an acute meningitis which is apparently benign. Livingstone and co-workers [*16*] reported two cases and reviewed the earlier case reports. In their first case headache, stiff neck, nausea, vomiting and photophobia developed within fourteen hours following spinal

anesthesia. There was marked lymphocytosis and elevation of the protein content of the cerebrospinal fluid. On the fourth day the patient was asymptomatic. Their second case was similar. Cultures of the cerebrospinal fluid including guinea pig and mouse inoculations, were sterile in both cases. The authors called this complication "chemical meningitis" but suggested no specific chemical agent. Rendell [*17*] encountered seven similar cases, in six of which stiff neck, headache, vomiting and lethargy developed within twenty-four hours following the administration of spinal anesthesia, with recovery in two to three days. A polymorphonuclear leukocyte response in the cerebrospinal fluid was observed in all seven patients. On the premise that inadequately washed syringes contaminated by disinfectants were a prime cause of postspinal anesthesia aseptic meningitis, she obtained syringes which had been stored in a phenolic disinfectant solution, washed them with "exaggerated care," and injected a spinal anesthetic in two patients. In one patient headache and low grade fever developed with no other symptoms or signs; however, the cerebrospinal fluid showed "a large number of polys." Symptoms and fever were not recorded in the other patient but the cerebrospinal fluid contained over 2,000 "polys" and the protein was elevated.

Hurst [*18*] has reported experimental studies in which monkeys were injected intrathecally with different concentrations of various chemicals including cationic quarternary ammonium compounds, anionic detergents, non-ionic detergents and various disinfectants including phenol. He produced pathological changes similar to those described by others following the use of detergents. However, he reported that such changes occurred only when rather high concentrations of the chemicals were injected. Despite the failure to produce an experimental acute meningitis in monkeys with trace amounts of phenolic disinfectants, the introduction of trace quantities of such agents into the subarachnoid space of human subjects seems to produce an acute benign purulent meningitis which is not followed by progressive neurological changes.

Because of the striking similarity between the patients we observed and the findings of Rendell, the technics in the care of spinal anesthetic equipment and anesthetic agents which were used in our patients were reviewed. Four of the patients were given 4 mg. tetracaine hydrochloride in 6 per cent dextrose solution distributed in 2 ml. ampoules. The ampoules were kept in their original cartons prior to being placed on the anesthetic trays which were then wrapped and autoclaved. At no time were the ampoules placed in disinfectant solutions. However, until shortly after the appearance of the first case, the syringes used for "saddle blocks" were cleansed according to technic utilized for "routine" syringes in the hospital. This consists of soaking syringes in a phenolic disinfectant solution (2.5 per cent Amphyl®), followed by washing and rinsing. In the Department of Anesthesia syringes for spinal anesthesia were kept entirely separate from other syringes and cleansed only in distilled water, alcohol and ether. This procedure was instituted by the Department of Obstetrics and Gynecology following the first episode of "chemical meningitis." However, many of the trays prepared by the older method were kept and periodically reautoclaved without opening the trays and rewashing. Also, it was possible that sporadic "breaks" in the procedure allowed a disinfectant-soaked routine hospital syringe to be included in the trays. In several of these patients the spinal anesthesia was technically difficult and it was quite certain that additional "routine" syringes were utilized. After reviewing the clinical and experimental observations relating to meningeal irritation following spinal anesthesia, we believe that the five patients herein described clearly represent chemical meningitis secondary to the intrathecal injection of phenolic disinfectant which contaminated the syringes used in the procedures.

If the problem of chemically contaminated syringes as used in various procedures is considered, the implications extend beyond spinal anesthesia. The seemingly unpredictable and capricious reactions to many intrathecal medications, including antibiotics, dyes and other agents may be explained in part by the use of chemically contaminated equipment. It is essential that equipment which is to be used for the administration of intrathecal materials must be handled according to procedures which are designed to eliminate completely exposure to chemical cleansing or disinfecting agents.

SUMMARY

1. Five patients in whom aseptic meningitis developed following spinal anesthesia are de-

scribed. Initially, each patient appeared to have an acute bacterial meningitis. In the majority, the onset of clinical meningitis occurred within fourteen hours following the administration of the spinal anesthetic. Recovery was prompt and no neurologic changes were encountered.

2. Review of the clinical and experimental observations relating to meningeal irritation following spinal anesthesia supports the impression that these patients had a chemical meningitis secondary to the intrathecal injection of phenolic disinfectants remaining on syringes following a cleansing procedure employing a phenolic disinfectant.

3. The necessity for more stringent procedures in preparing syringes which are to be used for the administration of intrathecal medication is emphasized.

Acknowledgment: We wish to express our appreciation to Dr. J. A. Pritchard for allowing us to follow and report these cases.

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Reviews

The Liver Cell*

Some New Approaches to Its Study

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WE will deal almost exclusively with those approaches to the understanding of the hepatic cell which we employ in our own laboratory: electron microscopy, specific staining methods for chemical constituents and enzyme activities, and the chemical analysis of subcellular fractions isolated by differential centrifugation of tissue homogenates. Autoradiography, the fluorescent antibody method, microspectrophotometry, tissue culture and other methods are largely omitted because we have had no direct experience with them. In some instances they have already made significant contributions to hepatic biochemical cytology.†

As Goddard [*47*] suggests, the prevailing intellectual climate as well as available technics profoundly influence scientific investigation. Virchow's enormous achievement in establishing a scientific basis for pathology resulted from his insistence on the cellular basis of disease [*136a*]. Because the means of studying cells were then so restricted, his concept of the liver cell (Fig. 1) included only a cell limit, a nucleus and a nucleolus.

Almost forty years later, when the first edition of Wilson's "The Cell in Heredity and Development" appeared, there were still large gaps in our knowledge of cells. The diagram of the generalized cell (Fig. 2) showed no mitochondria [*144*]. Chromatin then held the interest of investigators because it seemed clearly related to heredity and because it resisted much better than cytoplasmic particles the tissue fixatives then in use. Mitochondria had already been observed and described. Altmann's beautifully illustrated publication on cytoplasmic particles (which he considered "elementary organisms" or "bioblasts") appeared in 1890 and in 1882 Flemming had published his work on "fila." Even with Altmann's improvement of technics the mitochondria frequently were insufficiently delineated from other cytoplasmic granules. In 1892 Benda introduced the term "mitochondrion." Because of their overenthusiastic claims, perhaps, Benda, Meves and others succeeded in drawing the attention of cytologists to cytoplasmic particles. In the last edition of Wilson's book, in 1925, mitochondria are prominent in the diagram of the cell.

Almost ten years elapsed until Bensley and Hoerr [*9*] opened the era of intensive chemical study of isolated cytoplasmic particles, an era that was to bring together the fields of biochemistry and cytology. Although histochemistry had auspicious beginnings in the nineteenth century [*107*], it was Lison's work "Histochimie Animale" in 1936 which gave the field the impetus required for its modern development. By 1940 methods were published which were to make popular the demonstration of ribonucleic acid [*13,18*] and alkaline phosphatase activity [*49*], and with them, other specific staining procedures. Only in recent years, however, have

† To indicate some applications of these technics to the study of liver cells we mention the use of tritiated thymidine autoradiography to study the stimulatory effect of high levels of serotonin on cell division in the liver of young rats [*72*]; autoradiography, with radioactive chromium phosphate, in assessing the phagocytic efficiency of Kupffer cells [*29*]; microspectrophotometry, using the Feulgen reaction, to follow the ploidy changes with age [*1*]; tissue culture of liver cells to study humoral factors in regeneration of rat liver [*46*]; and the use of the Coons fluorescent-antibody technic to study gamma globulin formation in postnecrotic cirrhosis and hepatitis [*20*].

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staining methods for enzyme activities acquired validity at the intracellular level [*91a*]. Although electron microscopes were built in the early nineteen thirties and electron microscopy was an active field in the forties [*148*], it was not until 1949–1952 that improved methods of fixation, embedding and sectioning made this a versatile as well as powerful tool in biology and medicine [*128*].

In Figure 3 we attempt to show the chief features of a parenchymatous cell of the liver, as revealed by these newer biochemical and morphological technics. The diagram emphasizes the complexity of cell function and structure and the interrelatedness of cytoplasmic organelles. The focus today is on chemical and structural changes *inside* organelles such as mitochondria, endoplasmic reticulum, ergastoplasm, lysosomes, nucleus and cell membrane.

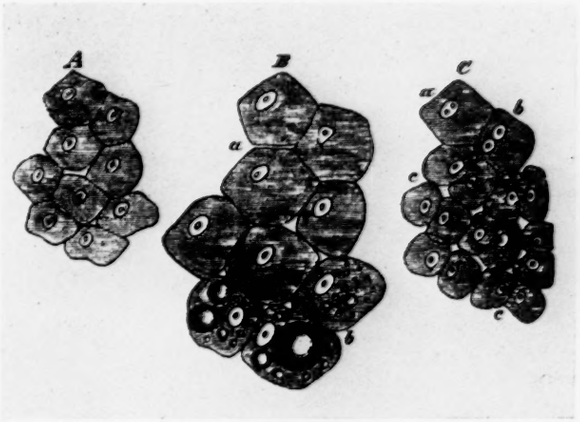


FIG. 1. Diagram of liver cells (from VIRCHOW, 1858 [*136a*]). A, normal liver cells. B, hypertrophied cells, with (b) and without (a) fatty degeneration. C, hyperplastic cells; a, with divided nucleolus; b, with divided nucleus; c, with divided cells.

THE POLARIZED NATURE OF THE HEPATIC CELL

The polarized nature of some hepatic cell functions is self-evident: materials are absorbed from the blood at the sinusoidal surfaces, and bile constituents are secreted at the surfaces exposed to the bile canaliculi. Correspondingly, at least some cell structures show polarized orientations.

Electron microscopy reveals that the absorptive and secretory surfaces of the liver cell are enormously increased in the microvilli, literally millions of finger-like extensions [*38,98,122*].*

Staining reactions suggest enzymatic differentiation of these two areas of microvilli. The order in which they are listed in Figure 3, and the number of plus marks in Figure 27, indicate the relative staining intensities resulting from the three phosphatase activities present at these surfaces, as seen in light microscope preparations of rat liver. By combining electron microscopy and staining reactions, Essner et al. [*37*] were able to localize the reaction product of adenosinetriphosphatase (ATPase) activity in the plasma membrane of the microvilli within the bile canaliculi; 5′-nucleotidase reaction product was

* Elias and Bengelsdorf [*33*] have stressed the importance to the mammal of the evolution of hepatic plates of one-cell thickness. They consider that one of its great advantages is the "more intimate contact of its cells with the blood stream and with the biliary systems."

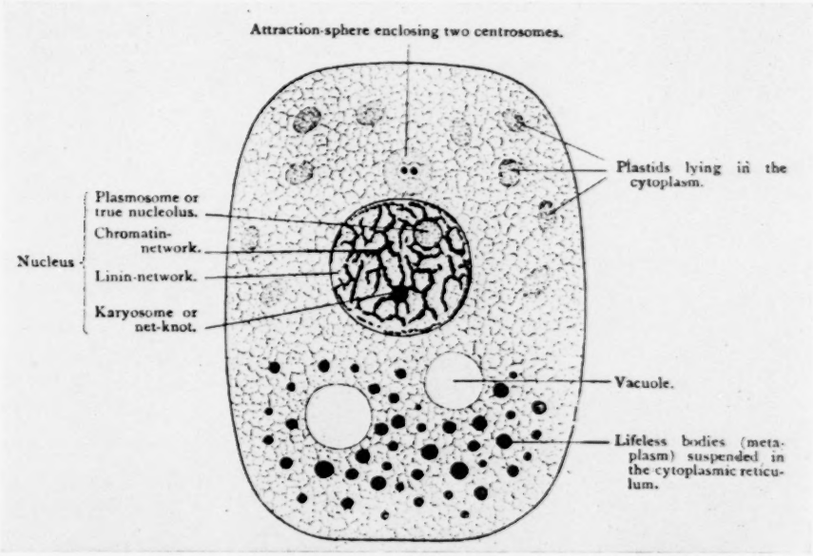


FIG. 2. Diagram of a generalized cell (from WILSON, 1896 [*144*]).

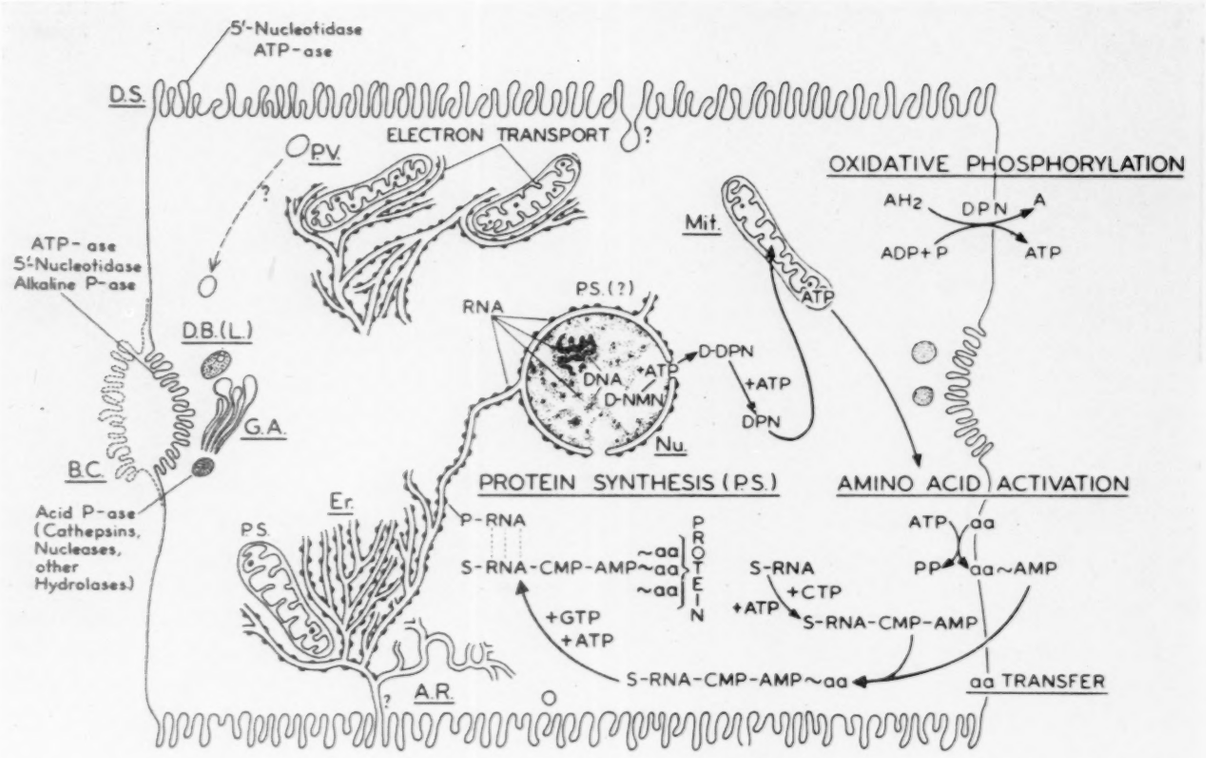


FIG. 3. A modern diagram of a parenchymatous cell of rat liver. Abbreviations: A, oxidized substrate; aa, amino acid; AH_2, reduced form of substrate; ADP, adenosinediphosphate; AMP, adenosine monophosphate; A.R., agranular reticulum; ATP, adenosinetriphosphate; ATP-ase, adenosinetriphosphatase; B.C., bile canaliculus; CMP, cytosine monophosphate; CTP, cytosine triphosphate; D-DPN, desamido diphosphopyridine nucleotide; DPN, diphosphopyridine nucleotide; DNA, deoxyribonucleic acid; D-NMN, desamido nicotinic acid mononucleotide; D.B., pericanalicular dense bodies; Er., ergastoplasm; G.A., Golgi apparatus; GTP, guanosine triphosphate; L., lysosomes; Mit., mitochondrion; Nu., nucleus; P., inorganic phosphate; P-ase, phosphatase; P-RNA, particle ribonucleic acid; PP, inorganic pyrophosphate; P.S., protein synthesis; P.V., pinocytosis vacuoles; RNA, ribonucleic acid; S-RNA, soluble ribonucleic acid (from Novikoff [*91a*]).

present in the sinusoidal and canalicular microvilli. Just how ATPase and other enzymes function in the cell membrane remains for future investigation. It is undoubtedly significant that in other tissues as well as in liver we have found that high levels of ATPase activity characterize cell membranes which are active in transporting molecules or in movement [*87*]. The suggested enzymatic differentiation of the absorptive and secretory surfaces of the liver cell probably has physiological significance, and alterations in such enzyme activities may be anticipated in disease states.

In the rat liver cell many mitochondria show a polarized orientation. As Figure 3 suggests, their long axes are oriented in sinusoid-canalicular directions. To what extent this distribution may be related to that of the endoplasmic reticulum is unclear (see discussion in Novikoff [*90*]). Such polarity is not restricted to rat liver. It has been described in the amphibian, Amphiuma [*109*] and in the mouse [*71*]; perhaps it is present in liver parenchymal cells of all species.

Significant morphological clues to a polarized uptake and transport of materials through the hepatic cell may be seen in the disposition of the vacuoles in the cell. These vacuoles are numerous at the sinusoidal aspects of the cell [*85*]. (Fig. 16.) The delimiting membranes of some vacuoles appear to be continuous with the cell membrane. (Fig. 16.) Frequently the vacuoles are arranged in linear arrays, consistent with movement in sinusoid-canalicular directions. (Fig. 16.) Both these features suggest that the vacuoles are pinocytosis vacuoles presumably transporting materials, taken up in packets from the environment, through the cell. Hampton [*52*] has demonstrated the uptake of injected col-

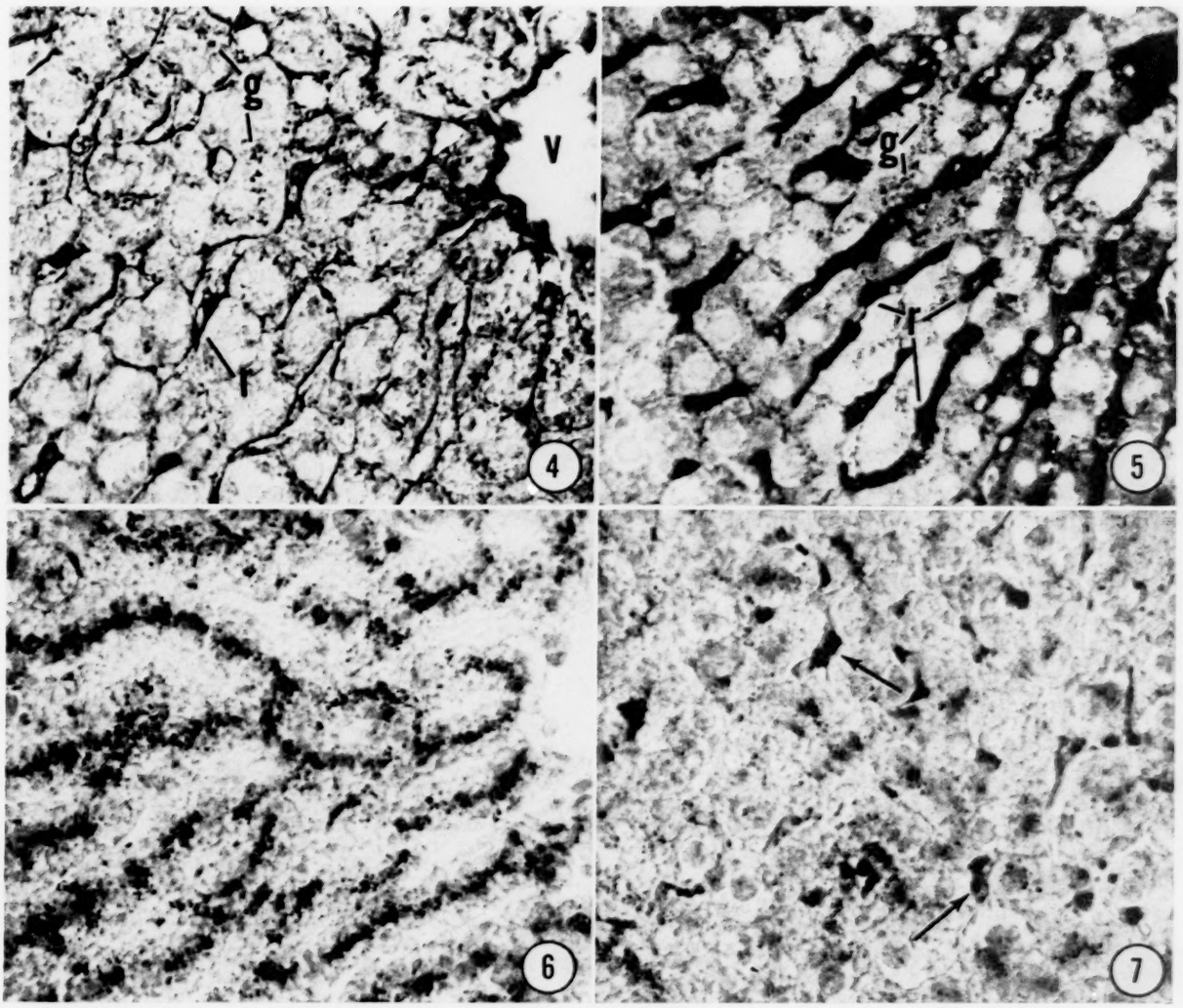


FIG. 4. Paraffin section, 3 μ, of liver of control rat fed dye-free diet [*76*] for one week; stained by the liver method described by Gatenby and Painter [*42*] following fixation in Aoyama's fluid. Magnification × 415. Note argyrophilic reticulum (r) lining the sinusoids. Central vein is seen at upper right (v). Within some cells, parts of the Golgi apparatus (g) may be seen.

FIG. 5. Paraffin section, 3 μ, of liver of rat fed 3′Me-DAB [*76*] for one week; processed in identical fashion and at the same time as that shown in Figure 4. Magnification × 415. Note increased amount of argyrophilic fibers (r); compare with Figure 16. Parts of the Golgi apparatus (g) may be seen. The central vein was adjacent to the upper right corner of the photograph.

FIG. 6. Frozen section, 10 μ, of liver of control rat fed dye-free diet for three weeks; fixed in cold formal-calcium [*5a*] and stained for acid phosphatase activity [*49*]; incubation time, forty-five minutes. Magnification × 415. Note the pericanalicular arrangement of the stained bodies (lysosomes).

FIG. 7. Frozen section, 10 μ, of liver of rat fed 3′Me-DAB for three weeks; tissue processed in identical fashion and at the same time as that shown in Figure 6, except that the incubation time was fifteen minutes. Magnification × 415. Because of the shorter incubation time, not all acid phosphatase granules (lysosomes) of the parenchymatous cells are seen. Even with longer incubation time, however, it was apparent that the lysosomes were not predominantly pericanalicular in distribution as in normal liver. Note the large numbers of active Kupffer cells and, within them, the intense staining of the large lysosomes or phagosomes (arrows).

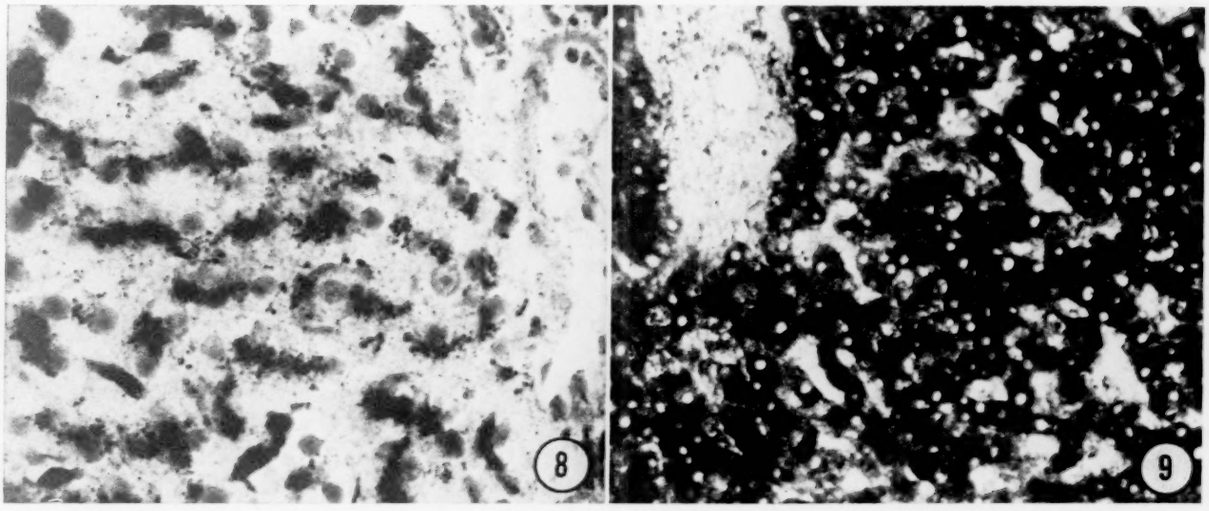


FIG. 8. Frozen section, 10 μ, of liver of rat fed 3′Me-DAB for six weeks; fixed and stained for acid phosphatase activity as in Figure 6, incubation time, forty-five minutes. Magnification × 415. The photograph shows the edge of an island of regenerating parenchymatous cells. Note the numerous acid phosphatase granules (lysosomes) in typical pericanalicular arrangement (*cf.* Fig. 6). Highly active Kupffer cells are numerous in the left half of the photograph.

FIG. 9. Frozen section, 10 μ, of human liver (seventy-two year old man), fixed overnight in cold formal-calcium and stained for succinic dehydrogenase activity, with nitro-BT as acceptor and phenazine methosulfate added to increase staining intensity. Incubation time, one hour. Magnification × 170. Note stained cytoplasm and unstained nuclei. The mitochondrial localization of succinic dehydrogenase is not apparent. It is just possible to see some of the intensely-colored formazan crystals artifactually deposited on small lipid droplets. The lightly stained connective tissue of the portal triad area is visible.

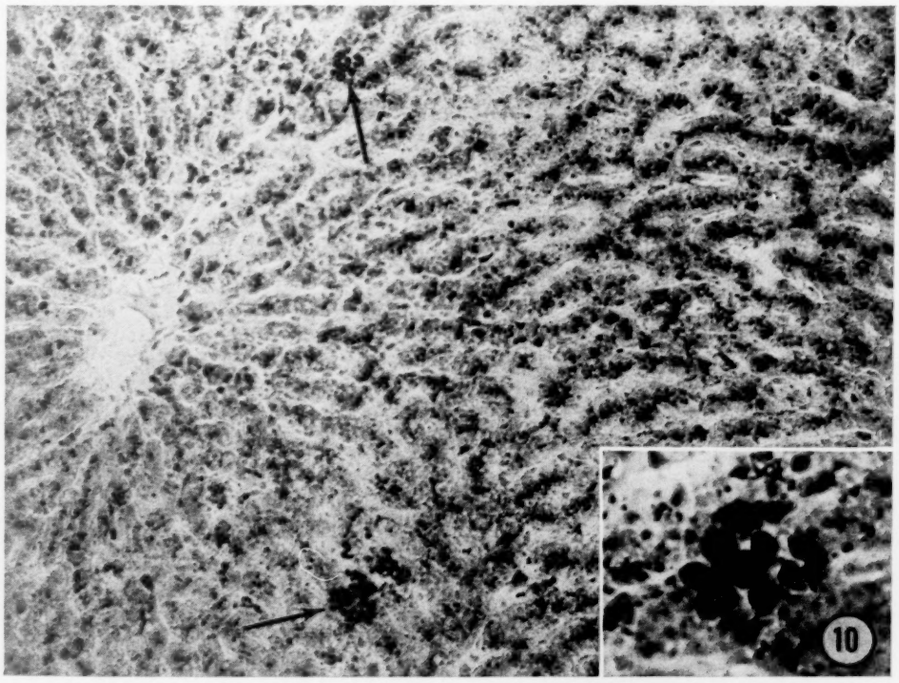


FIG. 10. Frozen section, 10 μ, of liver of rat twenty-four hours following ligation of the bile duct; stained for acid phosphatase activity [*49*], incubation time, forty-five minutes. Original magnification × 200. (Insert, magnification × 750.) Note enlarged lysosomes (cytolysomes [*91*]) in the two islands of necrotic cells (arrows), the upper one showing one cell (enlarged in insert) and lower one showing two cells.

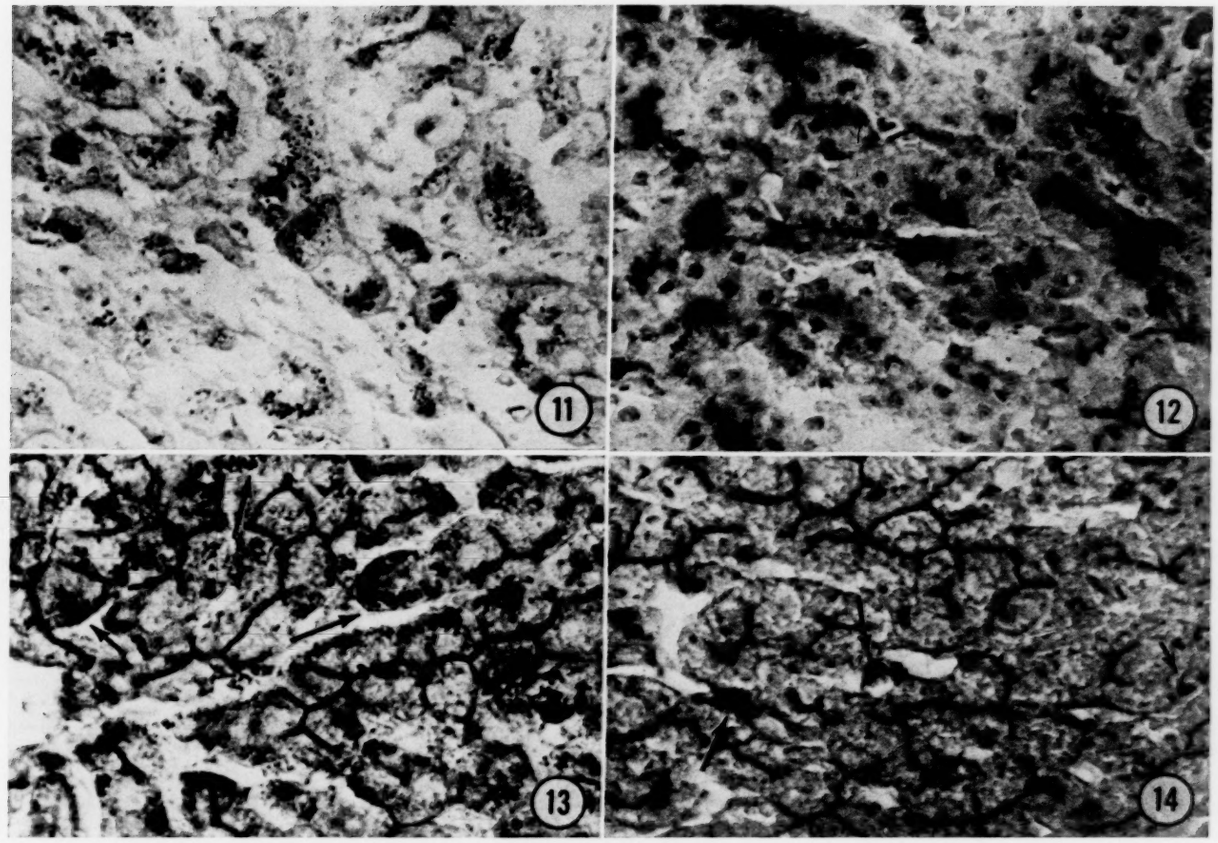


FIG. 11. Frozen section, 10 μ, liver of patient with hemochromatosis; fixed in cold formal-calcium [*5a*], one day after death, and stained for iron by Perls' reaction [*69*]. Magnification × 310. The hemosiderin granules show a general pericanalicular arrangement (*cf.* Fig. 5 [*36a*] of a case of acute hemolytic anemia; also [*43,44,55,73,118*]).

FIG. 12. Parallel section to Figure 11, stained for acid phosphatase activity [*49*]; incubation time, forty-five minutes. Magnification × 310. Note pericanalicular arrangement of acid phosphatase granules (lysosomes) within the parenchymatous cells. Compare with the distribution of the hemosiderin granules in Figure 11.

FIG. 13. Frozen section, 10 μ, of liver of homozygous normal Gunn rat, stained for ATPase activity [*137*] and for lipid, with oil red 0 [*49*]. Magnification × 500. Note small lipid droplets concentrated along the sinusoidal aspects of the parenchymatous cells (arrows). The bile canaliculi are delineated by their ATPase activity.

FIG. 14. Section of liver from homozygous deficient Gunn rat, processed in identical fashion and at the same time as that of Figure 13. Magnification × 500. Note that the parenchymatous cells lack the small lipid droplets and that the "fat-storing cells" (arrows) are filled with large lipid droplets (see text for a discussion of these cells).

loidal particles in such vacuoles. In our laboratory enzymatic proteins have been injected intravenously into rats [*96b*]. This induces a marked increase in the number of linearly arranged pinocytosis vacuoles. As we shall discuss in a later section, there is also an increase in the number of lysosomes in the cell and it is possible to demonstrate the presence of injected enzyme activities in pericanalicular lysosomes by means of staining reactions for these activities. The exact relation of pinocytosis vacuoles and lysosomes is currently being investigated.

The original suggestion [*92*] that in the liver parenchymal cells the pericanalicular "dense bodies" (Fig. 3) are lysosomes has found support in recent observations [*36*] which will be discussed subsequently, when we will also illustrate the usefulness of the simple staining method for acid phosphatase activity in the study of lysosomes. High levels of esterase activity are associated with pericanalicular granules, but the relationship of these granules to the acid phosphatase-rich lysosomes remains to be clarified [*136b*]. Another pericanalicular structure which may be related in some fashion to the lysosomes is the Golgi apparatus. Its disposition is readily revealed by the classic silver or osmium technics; careful study shows it always to be situated

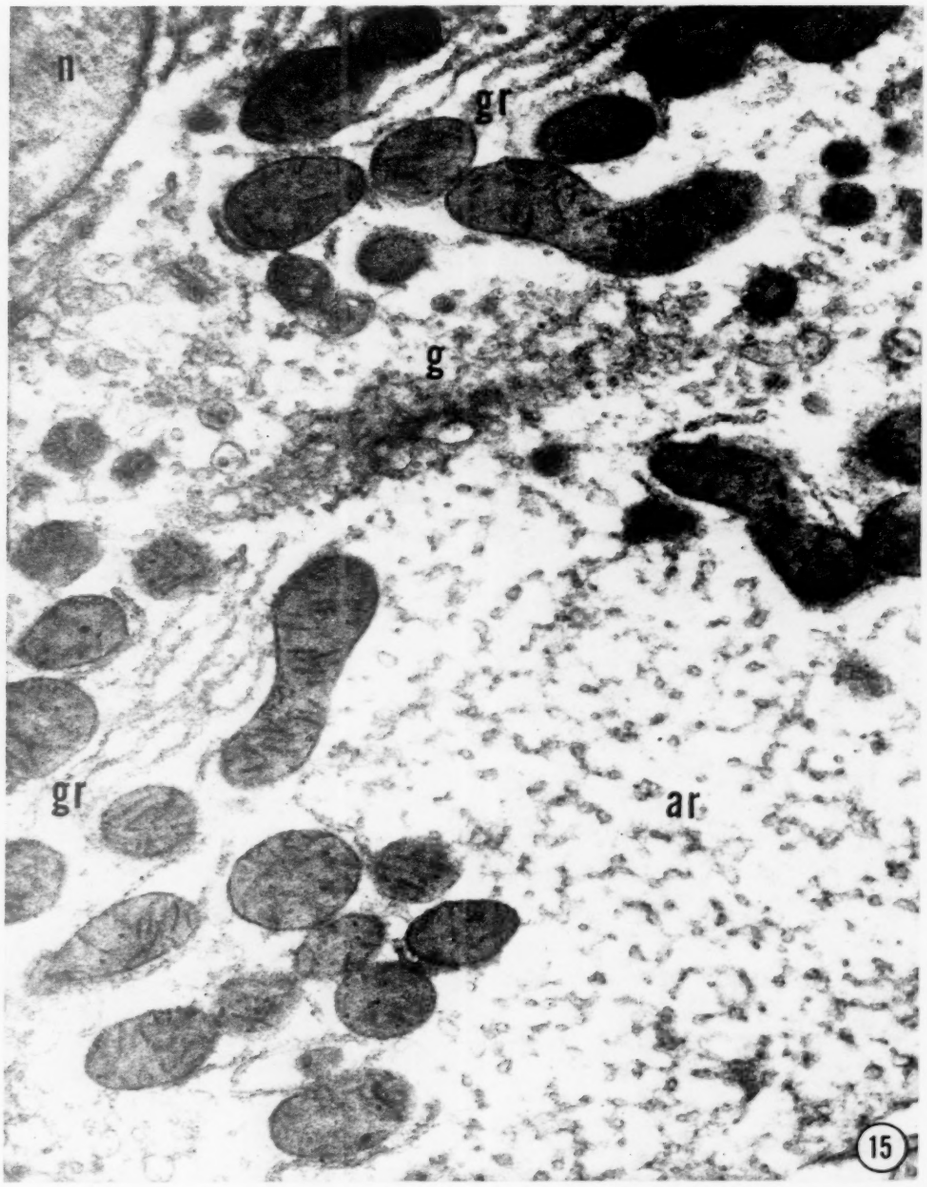


FIG. 15. Liver cell from homozygous deficient Gunn rat. Magnification × 19,000. A portion of the nucleus is seen at n. The small elements at g are probably part of the Golgi apparatus. Note that numerous small smooth-surfaced vesicles of the agranular reticulum (ar) occupy much of the cell volume. The granular reticulum (gr) is restricted to small areas among the mitochondria.

adjacent to the bile canaliculi [*95*]. Electron microscopy [*38,123*] demonstrates the Golgi bodies of the hepatic parenchymal cell to have the typical fine structure: parallel membranes and associated vacuoles (for two recent reviews of the Golgi apparatus see [*103*] and [*109*]). Figures 3 and 27 illustrate possible relationships among the pericanalicular structures [*84–86*].

Another sign of hepatic cell polarity is the frequent deposition of lipid droplets initially along the sinusoidal aspects of the cell [*114*]. (Fig. 13.)

MITOCHONDRIA

Figure 3 shows a few of the thousand or more mitochondria in an average parenchymatous cell of rat liver. Their characteristic fine structure is indicated diagrammatically: an outer smooth membrane separated by a short distance (about 100 Å) from an inner membrane which is folded

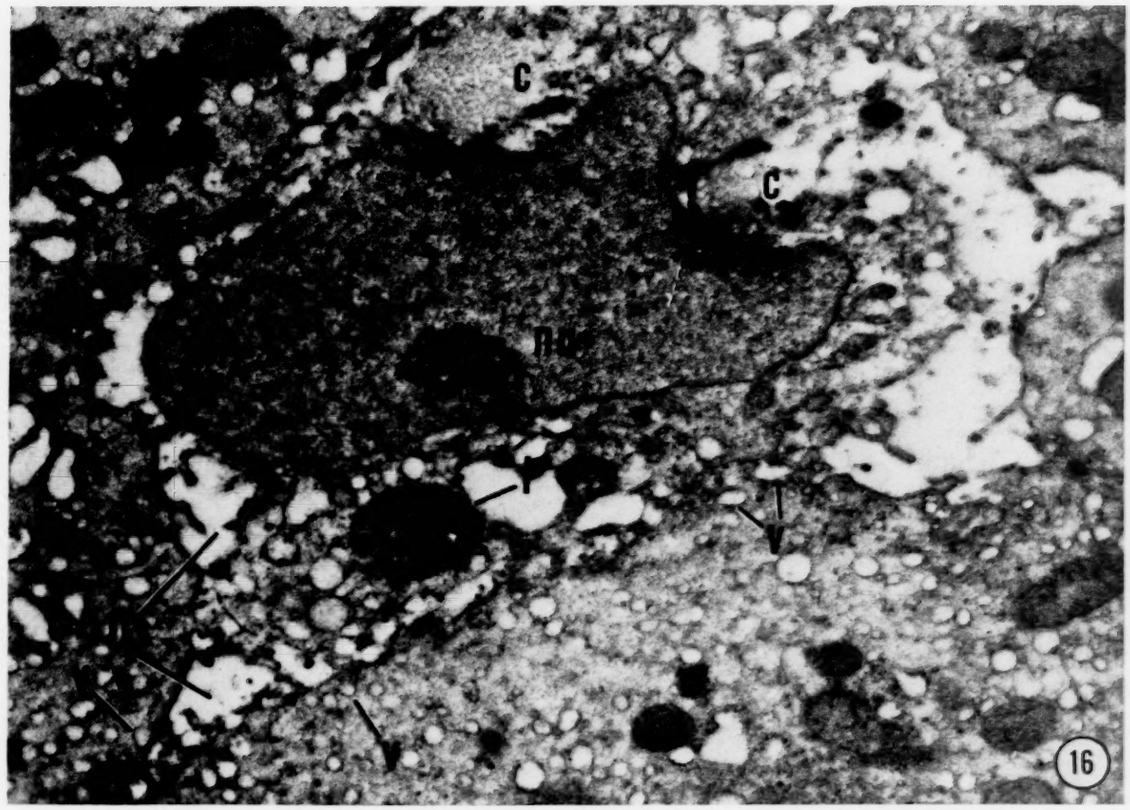


FIG. 16. Section through a sinusoidal region of liver in rat fed 3′Me-DAB for nine days. Magnification × 17,500. A large part of the sinusoid area included in this section is occupied by a Kupffer cell whose nucleus (n) fills most of the cell body. Within the nucleus is a nucleolus (nu). Note: (1) a large lysosome (l) in the Kupffer cell cytoplasm; (2) the intimate contact between the Kupffer cell and collagen (c) (argyrophilic reticulum) (the space seen between cell and collagen is an artifact of sectioning); and (3) numerous vacuoles both in the Kupffer cell and parenchymal cells; the membranes in some vacuoles (v) seem to be continuous with the cell membrane; in the lower cell the vacuoles appear to be arranged in linear arrays. The space of Disse is seen clearly at Ds.

into numerous "cristae" [*98*]. The cristae project, as baffles, into the inner compartment (matrix) in which are situated a few electron-dense spheres of unknown nature. Figures 15 and 17 show some of these features of mitochondria in rat liver cells, and Figures 19, 20 and 21 in human liver cells. Robertson [*120*] has discussed the possible nature of mitochondrial and other cell membranes.

An important achievement of differential centrifugation has been to establish the mitochondria as the chief and probably exclusive sites of oxidative phosphorylation. Oxidation of foodstuffs ($AH_2 \rightarrow A$) is coupled with the esterification of inorganic phosphate ($ADP + P \rightarrow ATP$). Thus some of the energy of foodstuffs, trapped in the terminal high-energy phosphate bond of ATP, becomes available to cell metabolism.

Increasing evidence is accumulating that the enzymes within mitochondria function as parts of an integrated system. "Enzymes are built into specific and precise structures and . . . their function depends to a large extent on organization. Enzymes do not operate in vivo as freely diffusible molecules. In a number of cases, parts of this structure have been isolated as units, ranging in complexity from the soluble proteins with polyenzyme function to particles with the high degree of organization encountered in the mitochondrion" [*51*]. In this system, phospholipid is apparently more than the cementing substance it was once considered. It seems to participate directly in the electron transport sequence, as coenzyme Q and combined with components such as cytochrome *c*. "The available evidence corroborates the view that the flavoproteins and cytochromes are connected

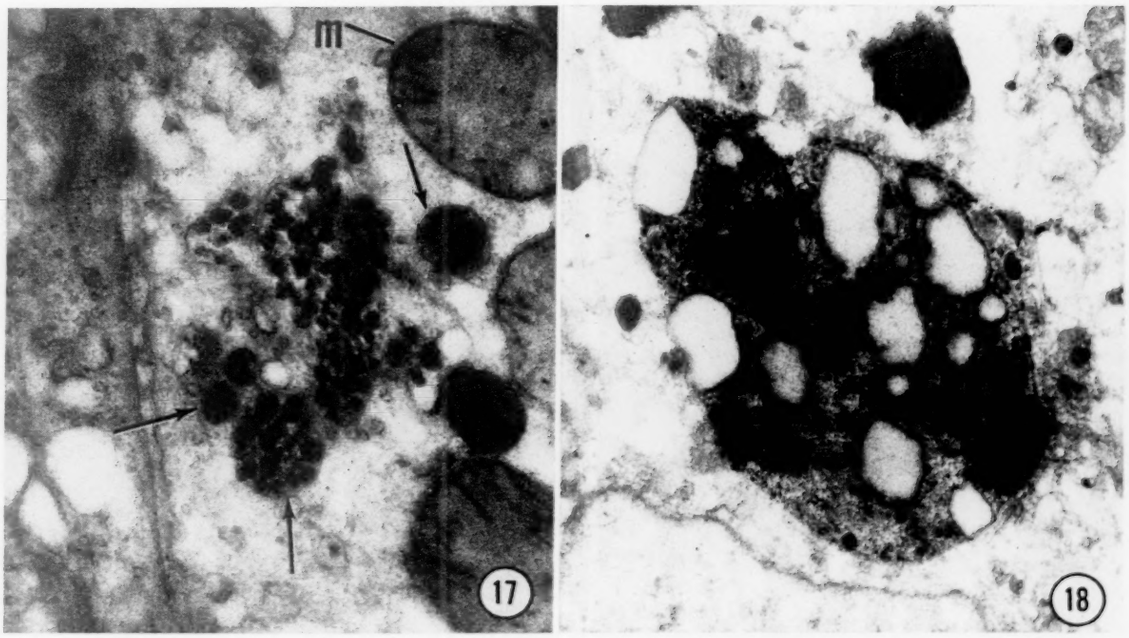


FIG. 17. Another area of the same liver as in Figure 16. Magnification × 24,500. Four mitochondria and a number of lysosomes are seen near the sinusoidal aspect of a parenchymatous cell. The lysosome membrane is seen to be "single" (arrows), in contrast to the "double" membrane of mitochondria (see at m). Compare these lysosomes with the "siderosomes" described by Richter [*118*] in the liver of ethionine-fed rats.

FIG. 18. A lysosome in a Kupffer cell in a rat fed 3′Me-DAB for four weeks. Magnification × 15,000. Note the complex nature of the lysosome, with vacuole-like areas of electron-lucid material, smaller areas of electron-opaque material, and very many electron-dense granules [*118,119*].

with one another through lipoproteins. In turn, these lipoproteins function as "restricted access highways' for the electrons 'being transferred through the mediation of compounds like coenzyme Q [*51*]" [*80*].

Cowdry [*23*] long ago stressed that mitochondria are much more sensitive indicators of cell damage than are nuclei. Because of their small size and because their demonstration required special cytological methods little attention was paid to them by most pathologists until electron microscopy brought them to a prominent position in cell pathology. It is now generally agreed that the vacuolar appearance of cells showing "cloudy swelling," in liver [*41*] as in tissues generally [*2,74*], is usually due to a swelling and rounding of the mitochondria, with rupture of cristae and loss of internal electron-absorbing material. (For references to electron microscopic and biochemical studies of altered liver mitochondria induced by treatments such as fasting, hormones and radiation, see [*90*]). Decreased oxidative phosphorylation has been noted in a number of such circumstances. Judah and Rees [*60*] have emphasized the loss of the coenzyme, diphosphopyridine nucleotide (DPN), which occurs in hepatic cells damaged by noxious agents such as carbon tetrachloride. Since DPN is vital to many biochemical reactions, including the oxidative steps of glycolysis and the tricarboxylic acid cycle, one can readily appreciate the biochemical lesions its loss could produce.

It is now possible to study the intracellular localization of oxidative enzymes in tissue sections, through the use of a new tetrazolium salt, "nitro-BT" [*79*]. However, with this salt as with the "MTT" of Pearse [*108*], there are still methodological difficulties such as diffusion of soluble dehydrogenases and the deposition of formazan in lipid. Caution is therefore advisable when drawing conclusions concerning mitochondrial dehydrogenases [*90,91a*]. For the work in our laboratory, we have preferred to use sections of formal calcium-fixed tissue rather than unfixed cryostat sections, chiefly because of the much superior preservation of cytological structure. The enzymes linking oxidation of the reduced coenzymes, DPNH and TPNH, to tetrazolium reduction survive such fixation. In rat liver on the other hand, succinic dehydrogenase activity is lost in such fixation. For

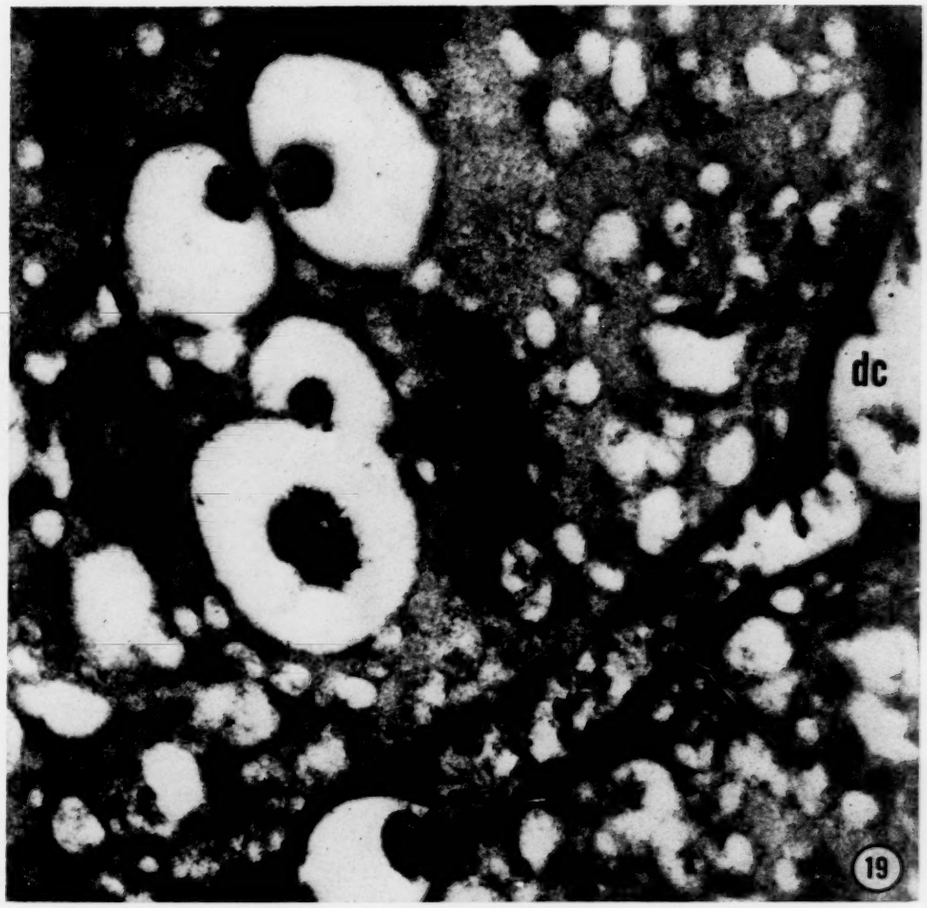


FIG. 19. Liver of forty-one year old male patient with obstructive jaundice; surgical biopsy specimen. Magnification × 27,000. Running diagonally across the lower right of the micrograph is a bile canaliculus. It shows normal microvilli in the region marked by a long arrow. Where the canaliculus is dilated (dc) the microvilli are reduced in number and size. Note that the electron-dense bile pigment deposits have a granular or roughly concentric fibrous form; see Essner and Novikoff [*36a*] for a discussion of this structure and its resemblance to the microspheroliths of bile. The deposits are within vacuoles. One, at least, of these is part of a lysosome (small arrow); if the others have similar caps of electron-dense material, these lie outside the plane of the section. The vacuolar space at the bottom, in which there is an accumulation of bile pigment with lamellated structure, may be a portion of dilated canaliculus or a vacuole continuous with the canaliculus. See text for a discussion of a possible role of lysosomes in bile transport in normal conditions and in obstructive jaundice.

some reason this apparently is not true of human liver. Figure 9 shows a frozen section of a needle biopsy of human liver, following overnight fixation, and incubation with nitro-BT and succinate as substrate.

The use of DPNH or TPNH as substrates (or of systems which generate them) in the tetrazolium procedure, when applied to liver sections, leads to widespread formazan deposition that obscures the stained mitochondria. This is due to the abundance of endoplasmic reticulum (*vide seq.*) in these cells. That this reticulum and its associated structures possess electron transport enzymes is indicated by staining results [*85,91a*] and by biochemical assay of isolated microsome fractions [*28,94*] which are largely derived from the reticulum.

ENDOPLASMIC RETICULUM; ERGASTOPLASM

One of the most unique contributions of electron microscopy to the understanding of the liver cell has been the description of a system of submicroscopic tubules and flattened vesicles (cisternae) coursing through the cytoplasm

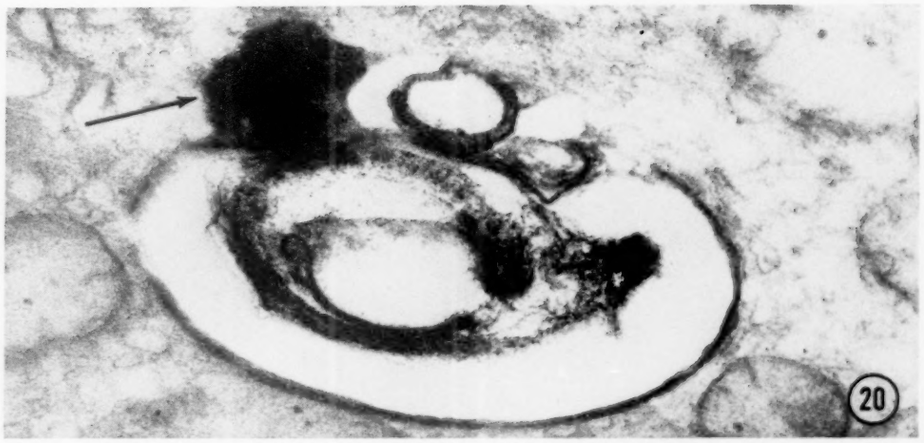


FIG. 20. Bile pigment deposit, from same liver as in Figure 19. Magnification × 29,000. Note the complex laminated and granular nature of the accumulation within a vacuole. The vacuole appears to be part of a lysosome. The arrow indicates portion of the lysosome with electron-opaque bodies.

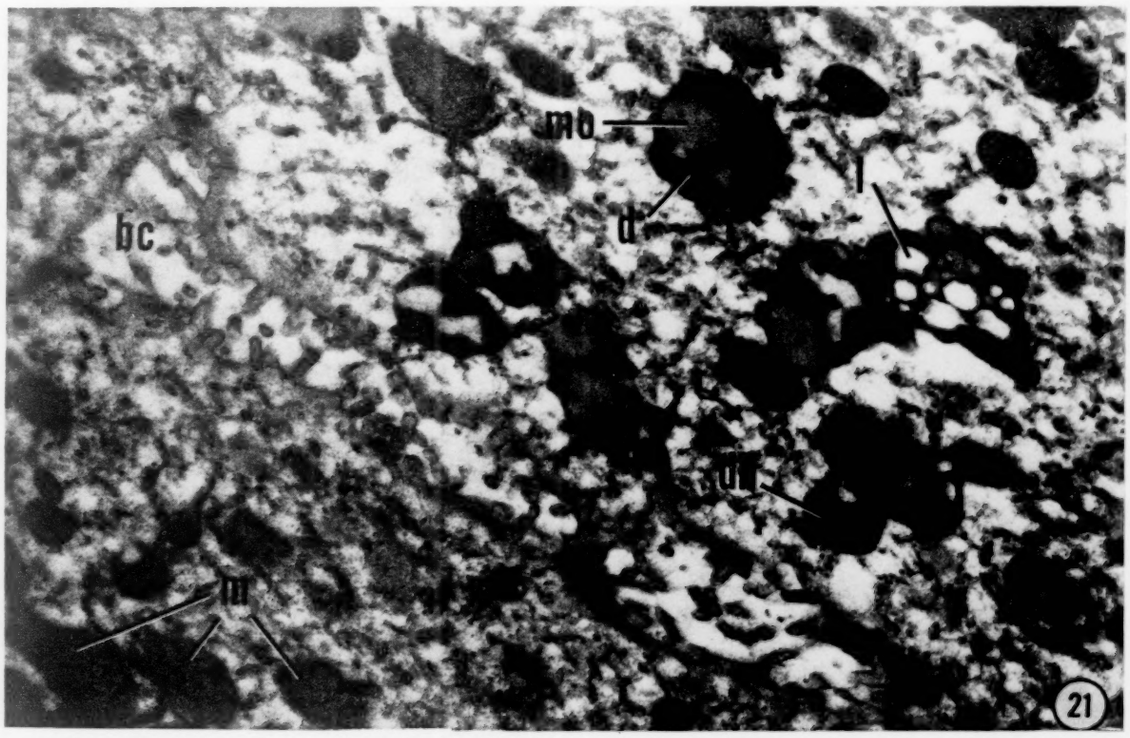


FIG. 21. Liver of seventy-two year old male patient, needle biopsy specimen. Magnification × 20,000. Apparent transitions from pericanalicular dense bodies (db) to large lipofuscin granules are described more fully in Essner and Novikoff [*36a*]. Note bile canaliculus (bc) with typical microvilli; mitochondria (m); and the three electron opacities within the lipofuscin granules: the peripheral electron-opaque material (d), the vacuole-like material of moderate opacity (mo) and the inner electron-lucid areas (l). See Figure 23 for ferritin-like granules in the altered dense body.

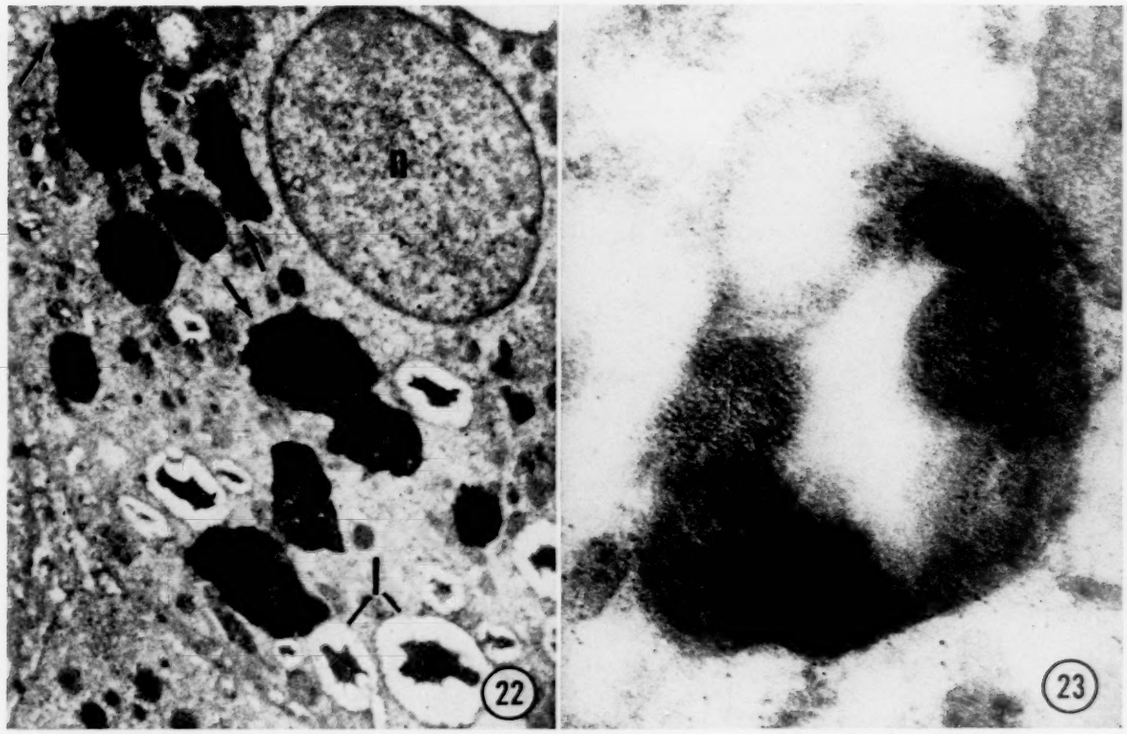


FIG. 22. Liver of sixty-five year old female patient with chronic idiopathic jaundice (Dubin-Johnson syndrome). Magnification × 9,800. Note nucleus (n), lipid droplets (l) and pigment granules. Within the latter, vacuole-like areas of moderate electron opacity may be seen (arrows), but electron-opaque material is more widely distributed than in the large lipofuscin granules. (Fig. 21.) The small electron-lucid areas in the pigment granules may be artifact.

FIG. 23. Transformed pericanalicular dense body in same liver as in Figure 21. Magnification × 70,500. Note ferritin-like electron-opaque particles, in peripheral lobular areas such as those in the large lipofuscin granules. (Fig. 21.)

[*99,115*]. Most investigators are agreed that the membranes seen in electron micrographs are sections through this continuous system, the endoplasmic reticulum. In the hepatic parenchymal cell this reticulum appears in at least two forms, "granular" and "agranular."

The *granular reticulum* or *ergastoplasm* is characterized by the presence of electron-opaque granules on its surface. Current opinion associates most of the cytoplasmic high molecular weight ribonucleoprotein with these granules which are referred to as ribonucleoprotein particles, or more recently, as "ribosomes." The particle RNA is thought to be the site of amino acid incorporation into protein. (Fig. 3.) When one reflects that all the serum albumin and some of the globulins, including fibrinogen, are produced in the liver [77], the signal importance of this system is evident. The *basophilia* of the ergastoplasm is considered to be due to the high RNA content of the particles. Noted as early as 1950 by Dalton et al. [*24*], in most liver cells the ergastoplasm is concentrated into restricted irregular areas (mostly as parallel arrays of cisternae) and thus in the light microscope the cytoplasmic basophilia appears as irregular clumps [*24*].

The *agranular (or smooth) reticulum* in normal liver is scantier than the granular form [*99,116*]. However, it may have great importance, as indicated by Porter and Bruni [*116*], who suggests an intimate role for it in glycogenesis or glycogenolysis. The suggestion is based on the finding of such reticulum in the glycogen areas—where newly-discovered "glycogen units," with a definite fine structure, are concentrated—and on the replacement of the glycogen units by hypertrophied agranular reticulum observed in rats fasted for forty-eight hours or longer.

It is from the endoplasmic reticulum and associated ribonucleoprotein granules that the so-called microsomes of liver are largely derived

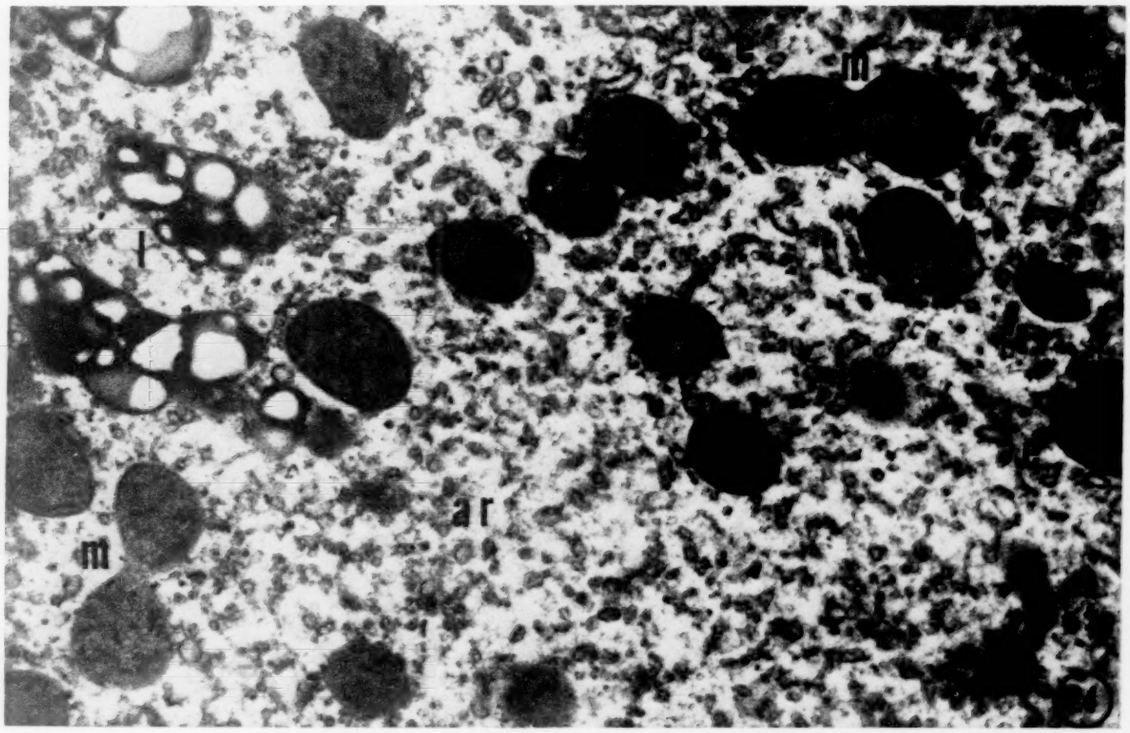


FIG. 24. Liver of fifty-two year old male patient with Gilbert's syndrome of the enzymatic type (deficient hepatic glucuronyl transferase activity [*3b*]. Magnification × 17,000. Note: (1) the scarcity of ergastoplasm, much of which appears in section as small fragments (e), (2) the wide area of agranular reticulum (ar), (3) several large lipofuscin granules (l), and (4) the mitochondria, some of which (m) have an appearance consistent with division. The section was stained with uranyl nitrate and lead hydroxide according to Watson [*14b*].

[*82,102*]. The manner in which the reticulum tubules and cisternae are fragmented is not yet understood. Also to be elucidated is the extent to which structures like microvilli contribute to the microsome fractions.

Continuities between agranular and granular reticulum have been described in the liver cell [*99,116*]. Continuity with the Golgi membranes, as found in other cells [*99*], has not been described in liver cells, nor has continuity between the endoplasmic reticulum and the outer cell membrane, or plasma membrane, been demonstrated. (Fig. 3.) Indeed, such continuity has been claimed in only a few cells [*34,83,99*], and it remains to be seen if this is a general feature of cells. At the other end, the endoplasmic reticulum may be considered, as Palade [*99*] has suggested, to be enlarged into a kind of moat enclosing the nucleus. The outer of the two nuclear membranes, like the ergastoplasmic parts of the reticulum, is studded with ribosomes.

Palade [*99*] was the first to speculate concerning the physiological implications of this continuous network of membrane-bound cavities coursing through the cytoplasm. He suggested that it constitutes a kind of submicroscopic circulatory system with an enormous area of membrane separating the fluid phase within from the solid phase (cytoplasm) without. However, even if the material enclosed within the membranes were more rigid than fluid, it still would be possible to conceive of transport of materials by the membranes, in the fashion suggested by Bennett [*8*]. One of us has speculated elsewhere [*83*] about the simple endoplasmic reticulum (intracellular channels) of a transplantable rat liver tumor cell, in which it appears to be continuous with both plasma membrane and outer nuclear membrane.

Early electron microscopic studies showed the endoplasmic reticulum of liver cells to respond rapidly to experimental procedures, as was to have been expected from the changes in basophilia observed with light microscopy. The disappearance of ergastoplasm from wide areas of the cell after fasting [*10,38,116*], partial hepatectomy [*11a*] or administration of carbon tetrachloride [*11a*] has been described. Bernhard

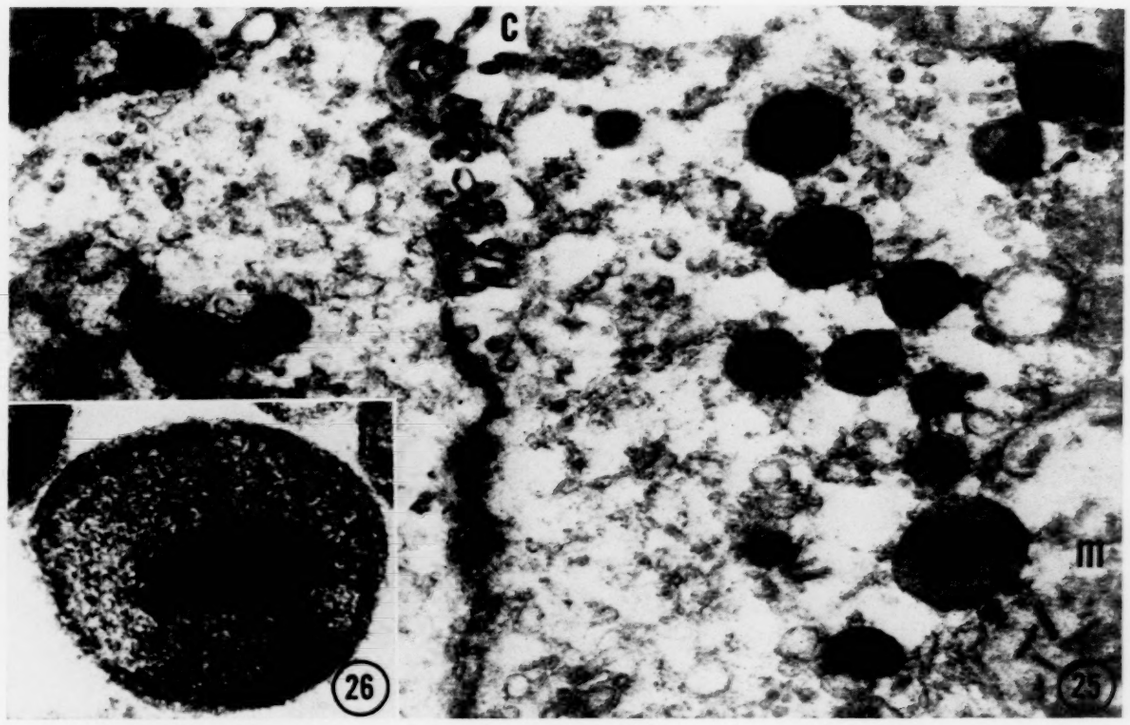


FIG. 25. Liver of a rat fifteen minutes after the infusion of conjugated bilirubin was begun. Magnification × 27,000. Note the numerous microbodies some of which possess more or less centrally-located areas of high electron opacity. One such microbody is enlarged in Figure 26. The bile canaliculus is at c and some swollen mitochondria are at m.

FIG. 26. A microbody from the liver shown in Figure 25. Magnification × 55,000. The electron-opaque area is seen to consist of a crystalline-like array of alternating dark and light material.

and Rouiller [*11a*] reported that ergastoplasm always reappears in intimate contact with mitochondria when fasting animals are re-fed, or when regeneration occurs following partial hepatectomy or administration of carbon tetrachloride. Fawcett [*38*] noted aggregates of agranular reticulum in fasted animals; Porter and Bruni [*116*] have recently described them in greater detail.

The endoplasmic reticulum of rat liver cells appears to be the target structure for the toxic carcinogenic azo dye, 3′-methyl-4-dimethylaminoazo-benzene (3′Me-DAB). Porter and Bruni [*116*] found that within two days of dye feeding the parallel arrays of ergastoplasm become disorganized and the ribonucleoprotein particles become detached and reduced in number. The amount of agranular reticulum increases greatly in the areas normally containing glycogen, and by eleven days of dye feeding it comes to replace virtually all the glycogen. The authors' speculations are of interest: " . . . it is suggested that the dye is metabolized within the elements of the smooth reticulum and that a major derivative of this metabolism is bound by certain proteins within the membranes of the vesicles. This binding, it would seem, serves to inhibit an enzyme or enzymes involved in glycogen storage. The resulting toxic effects leads eventually to the destruction of the liver cell. . . . Presumably, in carcinogenesis, the azo dye destroys all liver cells that differentiate for glycogen storage and conversely does not affect any mutant cells which lose the capacity for glycogenesis. Thus the dye serves to select abnormal forms which continue to proliferate and appear as tumors."

As already indicated, Porter and Bruni [*116*] have found a similar hypertrophy of agranular reticulum in the glycogen areas of fasting rats. We have found a parallel situation in the liver of an interesting strain of rats. In the Gunn strain, homozygous deficient animals are jaundiced due to a congenital inability to conjugate bilirubin with glucuronic acid. This inability, as in parallel human situations (physiological

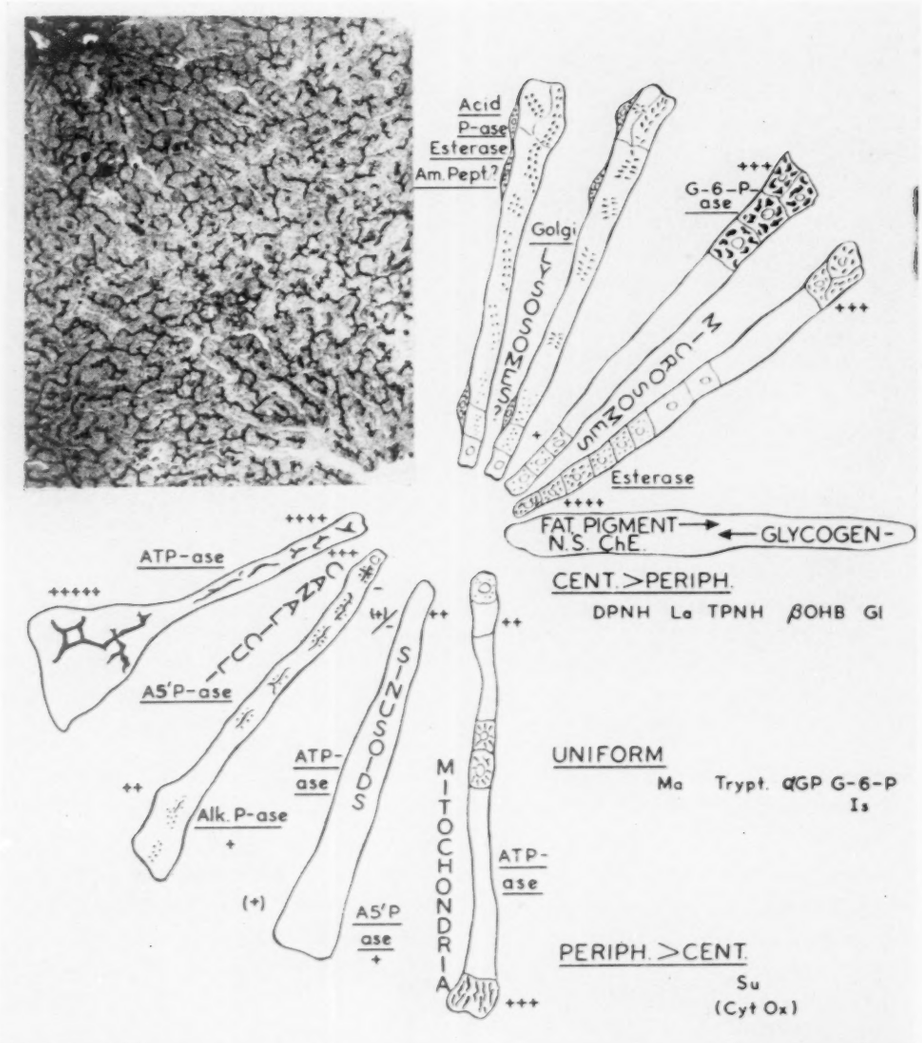


FIG. 27. Diagramatic representation of a hepatic lobule of the rat (from Novikoff [*86*]). In the upper left quadrant, a photograph shows a portion of the hepatic lobule stained to demonstrate ATPase activity in the bile canaliculi (from [*93*]). The quantitative differences between centrolobular cells and peripheral cells are indicated by plus marks (+) and by schematic representation of the cytologic structures. In the lower right corner, the substrates are arranged, from left to right, in decreasing order of staining produced with tetrazolium (nitro BT). Kupffer cells are indicated where they contain high levels of the enzyme activities or large numbers of the cytological structures drawn. The diffuse cytoplasmic staining obtained with unfixed frozen sections when 5′-nucleotidase activity is visualized is more intense (++) in the centrolobular area than peripherally (+). It has not been included in the diagram since we have not established whether this represents cytoplasmic (microsomal?) activity or diffusion (enzyme?) from the sinusoidal aspects of the cell. Abbreviations: ATP-ase, adenosinetriphosphatase; A5′P-ase, 5′-nucleotidase; Alk. P-ase, alkaline phosphatase; Am. Pept., aminopeptidase; Acid P-ase, acid phosphatase; G-6-P-ase, glucose-6-phosphatase; cent., centro-lobular cells; periph., peripheral cells; DPNH, reduced diphosphopyridine nucleotide; TPNH, reduced triphosphopyridine nucleotide; DPN-linked oxidations; La, lactate; β OHB, betahydroxybutyrate; Gl, glutamate; Ma, malate; αGP, alpha-glycerophosphate; and TPN-linked oxidations; G-6-P, glucose-6-phosphate, Is, isocitrate; and Su, succinate; Cyt Ox, cytochrome oxidase.

jaundice of newborn and premature infants, some forms of Gilbert's syndrome, and the Cragler-Najar syndrome), involves a deficiency of a transferase enzyme which normally is associated with the microsomes [*3*]. It may be of significance, therefore, that in many cells of one such jaundiced rat, we found extensive areas of agranular reticulum similar to those described in animals fed azo-dye. (Fig. 15.) In our first study of Gilbert's syndrome, we found many cells with wide areas of agranular reticulum as well as small fragments of granular reticulum. (Fig. 24.)

We will briefly refer to studies of altered liver cell function in which the endoplasmic reticulum appears to be involved and in which technics of biochemical cytology have made interesting contributions. The first relates to one type of glycogen storage disease (von Gierke's disease). Biochemical studies by the Coris [*22*] had indicated that in this disease there is a deficiency of the microsomal enzyme, glucose-6-phosphatase. This was confirmed by Chiquoine [*19*] employing a histochemical technic for the enzyme. The second example also deals with glucose-6-phosphatase. Recknagel [*117b*] finds this microsomal enzyme to be depressed by feeding carbon tetrachloride to rats before alterations occur in the activities of lysosomal or mitochondrial enzymes. The third example deals with liver regeneration. Glinos [*45*] has combined his observations on the changes in cytoplasmic basophilia following partial hepatectomy with evidence from tissue culture, parabiosis and other experiments. His hypothesis is that liver growth during regeneration, as in development, is self-regulated by the plasma proteins produced in the liver which are considered to inhibit liver cell division. The last example deals with the synthesis of cholesterol. It will be of great interest to see morphological studies on the livers of rats treated so as to elevate or suppress cholesterol biosynthesis [*15*]. That the ergastoplasm may be involved is suggested by the decisive role which the isolated microsomes play [*15*]. In the past few years the intermediates in cholesterol biosynthesis from acetate have been identified [*12,111,147*]. Acetate is "activated" (i.e., converted to acetyl-coenzyme A) by enzymes localized in the "supernatant fluid" isolated from liver. According to Bucher [*15*], the enzymes for converting acetyl-coenzyme A, through mevalonic acid and squalene, to cholesterol are bound to the microsomes. Some investigators find that the enzymes for converting mevalonic acid to squalene are recovered wholly or in part in the supernatant fluid, but Bucher believes that they are separated from relatively loose bonds to the microsomes during fractionation of the tissue.

LYSOSOMES

One of the most interesting consequences of the differential centrifugation method applied to liver homogenates has been the discovery by de Duve and his colleagues [*26*] in Louvain of a new class of cytoplasmic particles, the lysosomes. Until recently the lysosome was a concept deduced from biochemical analysis of isolated cytoplasmic particles [*28*]. In essence, de Duve et al. found that a number of hydrolytic enzymes, all with acid pH optima, could be concentrated in a small subcellular fraction which sediments between the usual mitochondrial fraction and the microsomal fraction. Eight such enzymes are now known: acid phosphatase, acid deoxyribonuclease, acid ribonuclease, cathepsin(s), β-glucuronidase, arysulfatase(s), phosphoprotein phosphatase, and β-N-acetyl glucosaminidase. The substrates for these enzymes include virtually all important chemical constituents of the cell. These hydrolytic enzymes are presumed to be enclosed within a lipoprotein membrane which restricts the physical freedom and the substrate-accessibility of the enzymes. A variety of *in vitro* treatments will solubilize all these enzymes simultaneously. *In vivo* changes in the relation of enzymes and substrates were suggested by de Duve [*26,27*] for phagocytosis, pinocytosis, and various lytic processes occurring in physiological and pathological circumstances.

A provisional link between the lysosomes and the pericanalicular dense bodies (to which we have alluded in our earlier discussion and which are included in Figure 3) was suggested by an electron microscopic study of the isolated fractions [*92*]. These bodies are characterized by outer membranes which in usual osmium tetroxide-fixed preparations appear single,* and frequently the presence of numerous electron-opaque particles presumed to be ferritin.

Considerable support for the link between these dense bodies and lysosomes has now come

* See Robertson [*120*] for a discussion of "single" and "double" membranes in such preparations. He considers the single membranes, about 75 Å thick, to consist of one bimolecular leaflet of lipid with its polar surfaces covered by monolayers of non-lipid material. Following potassium permanganate fixation, he finds such membranes to appear three-layered, with two dense lines, about 20 Å thick, bordering a lighter zone.

from a study of hepatocellular pigment deposition in human liver [*36a*], part of a broader study of lysosomes in which electron microscopy and cytochemical staining methods were central and biochemical assays supplementary [*91a*]. Essner and Novikoff [*36a*] were able to show that a variety of pigment granules in human liver possessed both the fine structure characteristics of the dense bodies and the acid phosphatase activity* of the lysosomes.

From electron micrographs, it is possible to construct a picture of transition from the typical pericanalicular dense body to a fully developed lipofuscin granule [*36a*]. (Figs. 21 and 23.) In the small dense bodies, acid phosphatase activity appears to be present throughout the granules. However, in the mature lobulated lipofuscin granule activity appears to be restricted to peripheral areas, apparently corresponding to the regions containing ferritin-like particles† [*31a,36a*]. (Figs. 21 and 23.)

The unknown pigment of chronic idiopathic jaundice (Dubin-Johnson syndrome) also appears to be deposited in the pericanalicular dense bodies [*31a,36a*]. When the pigment granules are not abundant they show a pericanalicular distribution. The granules possess high levels of acid phosphatase activity. Our knowledge of their fine structure is still limited. (Fig. 22.)

In a case of obstructive jaundice, biliary pigment accumulations were found within the pericanalicular dense bodies as well as in bile canaliculi and Kupffer cells. (Figs. 19 and 20.) Again, these pigment granules possessed high levels of acid phosphatase activity.

Finally, from the size, number and pericanalicular arrangement of hemosiderin granules (Figs. 11 and 12) it appears that iron may also be deposited in the pericanalicular dense bodies [*36a*]. Such pericanalicular arrangement has been reported in animal siderosis [*44,118*] and in African siderosis [*43,55*]. The Gillmans [*43,44*], like Mallory et al. [*73*] before them, believe this arrangement to be typical of early human hemochromatosis. Gillman and Gillman [*43*], describing the siderotic liver of the African pellagrin (which they consider hemochromatosis) wrote, "When the pigment is abundant, the localization is at first so precise that the distribution of the intralobular bile capillaries is accurately mapped out." Mallory et al. [*73*], in describing one of the figures, wrote, "The primary cell lesion of the liver in hemochromatosis: hemosiderin granules . . . packed in the liver cells close to the bile capillaries." Our Figures 11 and 12 are consistent with this description.

Gillman and Gillman [*43*], on the basis of light microscopy, and Richter [*118*], from electron micrographs, believe that the hemosiderin granules are intimately related to mitochondria. The Gillmans, in one place, speak of their origin "in intimate relationship with mitochondria" and, in another place, state that they have an "origin from mitochondria." Richter considers them altered mitochondria for which he proposes the term "siderosomes."

It is quite conceivable that with light microscopy the pericanalicular dense bodies would either go unnoticed or be confused with mitochondria. In a more recent electron microscopic study, Richter [*119*] points to the "single" nature of the membranes around several iron-containing bodies. His earlier electron micrographs seem too uncertain for the conclusion that the iron is deposited within mitochondria, particularly now in view of the parallelism we report in the size, number and distribution of iron-containing granules and acid phosphatase-rich granules or lysosomes [*36a*].

The most direct support for the link between dense bodies, including their altered forms in rat and human liver, and lysosomes has come from a recent study [*36b*]. Following overnight fixation in cold formal-calcium, small pieces of liver were incubated in the acid phosphatase medium [*49*], treated briefly with buffered osmium tetroxide and processed for electron microscopy in the usual fashion. Abundant deposits of enzyme product (lead phosphate) are restricted to the dense bodies of rat liver, to the peripheral portions of lipofuscin granules of human liver, and to the "microbodies" in the livers of rats infused with bilirubin.

Microbodies were first described in hepatic parenchymal cells by Gansler and Rouiller [*41*] and Rouiller and Bernhard [*124b*]. They studied regenerating hepatic cells of the rat after various experimental procedures. After partial hepatectomy, carbon tetrachloride poisoning, refeeding of starved animals, or several injections

* Enzyme activity was demonstrated in adjacent pieces of tissues from the same livers (needle and surgical biopsies). The tissues were fixed overnight in formal-calcium [*5a*] and incubated in the glycerophosphate-lead medium of Gomori [*49*] as free-floating 10 μ sections cut with the routine freezing microtome.

† This has been confirmed by direct electron microscopic study of the reaction product (lead phosphate). It is present only in the peripheral areas of the lipofuscin granules [*36b*].

of egg albumin, there is a marked increase of cytoplasmic particles 0.1 to 0.5 μ in diameter, delimited by single membranes and containing a finely granulated electron-opaque material. Their cores, usually homogeneous, sometimes show double membranes resembling cristae. These are considered by the authors as transitional forms between microbodies and mitochondria, indicating that mitochondria arise from microbodies. We would prefer to wait for more evidence than the occasional apparently double inner membranes before accepting so far-reaching a suggestion. We believe that most, if not all, microbodies are lysosomes. A transformation of lysosomes into mitochondria has not been encountered in any of the tissues studied in our laboratory.

Some comment is necessary regarding the evidence upon which the assertion is based that most, if not all, microbodies are lysosomes. In the liver incubated for acid phosphatase activity the accumulated reaction product obscures the fine structure of the microbodies, thus making only their size and distribution the basis of identification in these preparations. Although most microbodies are considerably smaller than mitochondria some size overlap does exist between large microbodies and small mitochondria. Another qualification relates to the electron-opaque deposits seen in livers (not incubated) of animals injected with bilirubin (Figs. 25 and 26) or ferritin (unpublished). Although we have seen no evidence that any of the bodies containing the deposits are mitochondria, the possibility exists that such deposits may have appeared in some mitochondria. These qualifications may alter our assertion only in a quantitative sense. Most, if not all, of the microbodies which we have seen are not small mitochondria. Most, if not all, show the acid phosphatase activity and single membranes characteristic of lysosomes. The electron-opaque material appears inside them within minutes following infusion of bilirubin or ferritin. This is consistent with the view expressed earlier that proteins and other injected materials may enter the cell in pinocytosis vacuoles which form at the sinusoidal surfaces. These vacuoles apparently move through the cell and accumulate near the bile canaliculi. It is possible that some pinocytosis vacuoles merge with or condense into lysosomes where some injected materials may be digested. Acid phosphatase-rich particles are present near the sinusoid and within the cytoplasm; these are more numerous following intravenous injection of the substances indicated. These particles are readily observed in thinner paraffin sections treated with saliva and stained by the periodic acid-Schiff procedure. In liver, as in several other tissues studied, some mucopolysaccharide is apparently concentrated in the lysosomes. This is true of the microbodies as of the lysosomes without inner electron-opaque deposits.

A role of pinocytosis vacuoles and lysosomes in the normal transport of bile constituents towards the canaliculi remains to be established. Bilirubin transport through the hepatic cell has recently been discussed by Cameron [*17*].

Under a variety of experimental conditions (bile duct ligation, carbon tetrachloride poisoning, and ingestion of 3′Me-DAB) there is a changed distribution of the acid phosphatase bodies (also the Golgi apparatus): fewer of them are pericanalicular and more are present elsewhere in the cell [*36,84*]. This may indicate a non-specific response to noxious agents, in which the entry and transport of materials via pinocytosis vacuoles and lysosomes is altered.

Lysosomes undergo pronounced changes in pathological conditions. It is often a simple matter to detect even a single cell in early necrosis by the marked enlargement of the lysosomes (cytolysomes [*91a*]). This is seen in Figure 10, from a section of rat liver twenty-four hours after ligation of the bile duct.

Acid phosphatase preparations may also be useful in locating nodules of regenerating parenchyma in some instances. The cells of such nodules, in the livers of rats fed carcinogenic azo dyes, show a marked accumulation of lysosomes in typical pericanalicular arrangement. (Fig. 8.)

We shall return later to consider the lysosomes of the Kupffer cells. We conclude this section by reference to the interesting work of Paigen [*97*] dealing with genetic influences on the localization of enzymes in lysosomes and other cell organelles. In the mouse, a single gene appears to affect both the concentration of β-glucuronidase in the liver and its intracellular localization. The ratio of lysosomal to microsomal activity is close to unity in the normal; it is about 3 in the mutant.

LIPID DROPLETS

The number of lipid droplets in liver cells is quite variable, depending upon dietary and other factors [*25,114*]. That it may reflect genetic influences is indicated by our observations with

the Gunn strain of rats. In the three homozygous deficient animals studied, but not in the three heterozygotes or three normal homozygotes, there are essentially no lipid droplets in the parenchymatous cells; but certain sinusoidal cells, more numerous in the peripheral areas of the lobule, are filled with large lipid droplets. (Figs. 13 and 14.) We believe these to be identical with the "fat-storing cells" of Ito [*59*] which have been described in a variety of species.* These cells are readily distinguished from the "inactive" lining cells of the blood sinusoids by their larger cell bodies and nuclei. They also can be differentiated from "active" Kupffer cells on the basis of reticulin-stained sections. Ito described the fat-storing cells as separated from the sinusoids by the argyrophilic reticulum. Our electron micrographs confirm this description. We also find these cells to differ from the active Kupffer cells in lacking both the enlarged lysosomes, with high acid phosphatase activity, and the high ATPase activity in the plasma membrane. It will be of interest to study the genetic or functional significance of their proximity to the lining and Kupffer cells. In the homozygous deficient Gunn rat, the Kupffer cells are essentially free of stainable fat.

In man, variable amounts of fat are found in the Kupffer cells of presumably normal liver [*40,68*]; in livers with vascular stasis, Fischer [*40*] found more fat in the Kupffer cells than in the hepatic cells; and Rössle [*121*] observed that in the diabetic liver the Kupffer cells were almost always filled with fat. In Gaucher's disease and Niemann-Pick's disease the Kupffer cells, like other reticulum cells, become laden with lipid to produce the so-called "foam cells" [*75*]. Both disorders are "inborn errors of metabolism" [*75*], and a strong familial influence has been described in both.

Many earlier investigators have concluded that in liver, as in other cells, lipid droplets originate from mitochondria. However, only the increased resolving power of the electron microscope will make it possible to draw unequivocal conclusions regarding such transformations. Such studies of liver are mentioned by Palade and Schidlowsky [*101*], Palade [*100*] and Porter and Bruni [*116*] in guinea pigs deprived of food, in cortisone-treated rats and in rats fed 3′Me-DAB. More complete publications deal with pancreas [*100*] and other organs. In no instance is there any evidence for conversion of mitochondria into lipid droplets. However, an intimate relation between mitochondria and lipid droplets is common in some circumstances. Palade [*100*] has speculated that the intimate contact reflects oxidation of lipid, mobilized from depots, by fatty acid oxidases of the mitochondria.

* In three additional homozygous deficient rats studied recently the fat-storing cells were evident in only one.

THE NUCLEUS

The three approaches—isolation of subcellular particles, cytochemical staining and electron microscopy—which have yielded so much information concerning cytoplasmic particles of hepatic cells have brought surprisingly little knowledge about their nuclei. The nuclear fraction obtained by differential centrifugation is sufficiently contaminated by cytoplasmic constituents to render difficult evaluation of the low levels of enzyme activities usually found in this fraction. The proportion of total enzymatic activity recovered in the nuclear fraction is rarely sufficiently high to permit cytoplasmic contamination to be disregarded. However, one such case involves the enzyme which catalyzes one step in the synthesis of DPN from nicotinic acid [*117a*]. (Fig. 3.)

Cytochemical staining methods permit the localization of substances like DNA in nuclei but those for enzyme activities are beset with difficulties such as nuclear adsorption of reaction product and enzyme (in phosphatase procedures) and the inability of nuclei to bind tetrazolium salts (in oxidative enzyme procedures).

Electron microscopy until now has yielded but little information concerning the genic and other materials of the nucleus. It has shed little light on the mechanisms of transmission of genetic information considered to pass from genic DNA, presumably via RNA, into the cytoplasm where most protein synthesis occurs [*14*]. The meaning of nuclear "pores" (Fig. 3) and other observations relating to nuclear-cytoplasmic relationships [*135*] remains to be established [*141c*].

Nuclear inclusions, found under a variety of circumstances [*65a*], are being studied by electron microscopy. Their pathophysiological interest [*65a,66*] remains despite the suggestion that in most instances the inclusions will be found, in fact, to be invaginated cytoplasm. Kleinfeld et al. [*63*] found that the hepatic nuclear inclusions of a patient with biliary cirrhosis and also of thioacetamide-treated rats were always enclosed within nuclear membrane. The inclusion, then,

was cytoplasm trapped within the invaginated nuclear membrane. Wessel [*143*] observed a similar situation in the livers of colchicine-treated mice. We find that in the livers of rats fed 3′Me-DAB clusters of lipid droplets may be present within the nuclei. Electron microscopy shows such clusters to be separated from the nuclear substance by a membrane, identical in appearance to and presumably an invaginated part of the nuclear membrane. In the cells of a transplantable mouse hepatoma and in enlarged liver cells of mice fed a bentonite diet, Leduc and Wilson [*65b*] found large intranuclear inclusions of cytoplasm separated from the nuclear substance by invaginated nuclear membrane. Luse et al. [*70*] found that lipid inclusions were present in the liver of the untreated fetus and of choline-deficient or cod liver oil-injected adult animals. These were "often surrounded by inward extensions of nuclear membrane. Our observations suggest that not only pores but also pinocytosis by nuclear membrane are involved in nuclear penetration."

It is well known that in diabetes mellitus the nuclei of human liver possess large amounts of glycogen [*114*]. One of the earliest reports of electron microscopy of tissue sections [*78*] dealt with such glycogen inclusions but due to the technical limitations of the time the presence of nuclear membrane around the inclusions cannot be judged in the published micrographs. Recently, Himes and Pollister [*56*] have studied the tadpole liver in which glycogen inclusions are always present in the nuclei. They found these to be truly intranuclear, without evidence of investment by nuclear membrane or of cytoplasmic RNA or protein within the nucleus. The same is true of the glycogen inclusion bodies described by Binggeli [*11b*] in the nuclei of chicken sarcoma cells; these nuclei also contain vacuole-like inclusion bodies.

BILE CANALICULI

It has been known since the early work of Gomori [*48*] that alkaline phosphatase preparations are useful for the study of bile canaliculi in tissue sections. More recently, Wachstein and Meisel [*136b,137*] showed that the use of ATP as substrate, in the presence of lead ions at neutral pH, yielded preparations which are superior for canalicular details (Figs. 13, 14 and 27). By such ATPase preparations changes in all canaliculi can readily be observed. Thus, following ligation of the bile duct in the rat and in obstructive jaundice in man, a loss of canalicular ATPase activity occurs throughout most of the lobule; only in the most peripheral parts of the lobules do the canaliculi retain sufficient activity to cause staining.* Electron microscopy [*123*] (also unpublished results of our own) shows that in the dilated canaliculi present in these circumstances the microvilli are much reduced in number and length (Fig. 19). We have also found that in human liver cells with a great many granules containing either lipofuscin or the pigment of chronic idiopathic jaundice one sees distinct discontinuities in the canaliculi in ATPase preparations [*31a*]. However, electron microscopy would be required to determine if this involves altered function only (decreased ATPase activity) or whether structure (canalicular microvilli) is also lost.

In the rat, after three weeks of feeding rats the dye, 3′Me-DAB, most of the canaliculi are no longer visible in ATPase preparations, as when the bile duct is obstructed experimentally or spontaneously. However, alkaline phosphatase preparations reveal that in both cases the canaliculi are still present; indeed, their alkaline phosphatase activity is considerably elevated [*89*].

Rouiller [*124a*] has suggested that the variations found in normal liver, both in width of canaliculus lumen and in structure of microvilli, may reflect functional differences among hepatic cells.

Electron microscopy has revived the question of direct continuity between bile canaliculi and sinusoids. There seems little question now that between the parenchymatous cell surface and sinusoidal lining cell there is a space—the space of Disse [*38,52,85,124*]. Granting that considerable departure from the living state is possible during removal of the tissue and in its fixation, and also that current methods of electron microscopy may leave much material unvisualized, the observation of well formed microvilli on parenchymal cell surfaces leads inescapably to the conclusion that the spaces do indeed exist. They are probably filled with plasma. General opinion [*38,52,105,124*] now holds that the lining cells do not form a continuous layer separating the Disse spaces from the sinusoidal blood; con-

* ATPase activity reappears in the bile canaliculi following removal of the obstruction. This had been observed in rats as part of a student experiment in our pathology course. Recently we observed a similar situation in man. The first of two biopsy specimens was taken when the bile duct was obstructed and the second three weeks following surgical removal of the obstruction.

trary opinion is in the minority [*4*]. However, it is not established whether this involves perforations in the extended lining cells or is due to spaces between mesenchymal-type cells [*52*].

Let us turn to the question of continuity of bile canaliculi and Disse spaces. Rouiller [*123*] concluded that such connections did exist, in normal rat liver as well as in animals with ligated bile ducts. In the latter, canalicular dilatation simply made the connections easier to detect. We have ourselves searched for such connections in rats with ligated ducts and in normal animals. We have encountered only one instance similar to that shown by Rouiller in his Figure 8. This was in an animal three days after ligation of the bile duct. However, we hesitate to conclude that even this instance represents the continuity for which we searched. We often find it difficult to differentiate the microvilli of the canaliculi from those of the Disse spaces. Secondly, we find that the sinusoids extend deep into adjacent liver cells, and these microvilli-lined extensions (which are narrow and triangular in shape) sometimes resemble canaliculi. In any event, if connections between canaliculus and Disse space exist, they are quite uncommon, even following obstruction of the bile duct in rats and in man. Hampton [*52*] also failed to confirm Rouiller's observations. Following retrograde injection of colloidal particles into the bile duct, masses of injection media could not be seen in the Disse spaces as would be expected if connections between the two were present.

If, as seems the case, direct continuity between canaliculi and Disse spaces is not common in obstructive jaundice then movement of bile into the blood (regurgitation) would probably involve intracellular transport. It is tempting to suggest that lysosomes may play a role in bile transport in this direction, as they may towards the canaliculi in usual circumstances. In human obstructive jaundice, bile pigment deposits are found within lysosomes. (Figs. 19 and 20). These pigment-containing lysosomes are not confined to peribiliary locales; indeed, some are close to the sinusoids and a few seem to be emptying into the sinusoids.

On the other hand, it is frequently held that bile regurgitation results from rupture of the canaliculi, permitting bile flow into the sinusoids. Gray [*50*] writes, "The canaliculi rupture at . . . the point at which the canaliculi emerge from the liver-cords, and the bile is returned to the blood-stream via the sinusoids." The 1926 paper of Klemperer et al. [*64*] shows a photomicrograph of a canalicular tear. However, Popper and Schaffner [*114*] consider it unlikely that such canalicular rupture is significant in regurgitation. In this connection it is relevant that ATPase preparations of normal liver show occasional canaliculi lying close to the sinusoids [*30,33,137*]. We have observed such approximations to be more frequent following ligation of the bile duct in the rat, as have Wachstein and Meisel [*138*]. Some kind of direct and rapid passage of bile into the sinusoids is established by the work of Hanzon [*53*]. By fluorescence microscopy the fate of injected uranin was followed in living rats with ligated bile ducts. The canaliculi become much dilated with uranin, and then the uranin flows abruptly into the blood. It is, however, not clear whether the flow is directly from the canaliculi or through injured parenchymal cells.

It is of interest that following partial hepatectomy of the rat the bile canaliculi show the same alterations as Wachstein and Zak [*140*] showed to occur when the bile duct was ligated in the rabbit. The canaliculi become distended and tortuous and they branch in remarkable fashion [*95,138*]; they also show increased alkaline phosphatase activity [*95*]. It would be of interest to study these reproducible changes with the electron microscope.

Contrary to what might have been expected from the studies of Elias and earlier work [*32*], electron micrographs fail to show a special wall in the bile canaliculi. Rather, the canaliculi are bounded only by the plasma membranes of adjacent cells [*38,98,123*]. (Figs. 19, 21 and 25.) Yet, canalicular fragments survive homogenization and are sedimented into the nuclear fraction [*95*], still retaining their phosphatase activities [*93,96*]. This is because adjacent cells are most firmly attached to each other along the canaliculi where there are "terminal bars" (thickened plasma membrane with electron-opaque material) and occasional interlocking stud-like processes [*38*].

BIOCHEMICAL INTERRELATEDNESS OF INTRACELLULAR STRUCTURES

As indicated elsewhere [*96a*], the most essential contribution of differential centrifugation to our understanding of the cell is so commonplace that we may overlook it. The intracellular localization of chemical substances, revealed by this

technic above all others, implies an *interdependence* of subcellular units.

Figure 3 includes a striking instance of metabolic interrelatedness among cell organelles; other examples are given by Lehninger [*6*]. As already indicated, one of the steps of DPN synthesis apparently occurs in the nucleus [*117a*]. Presumably some of this DPN is that which is utilized in oxidative phosphorylation within the mitochondria. The resulting ATP is utilized in a great many reactions within and outside the mitochondria. The enzymes catalyzing the so-called activation of amino acids appear to be situated in the soluble phase or supernatant fluid. In the soluble phase, too, are the enzymes attaching the nucleotides of cytidine and adenine to the so-called soluble ribonucleic acids. Through transfer to the nucleotides of specific ribonucleic acids, the amino acids are somehow transported to the ergastoplasm where, in the ribosomes, they are incorporated into protein. The enzymes involved are also recovered in the soluble fraction. Recent reviews of the sequence beginning with amino acid activation and culminating in polymerization of amino acids into protein are those of Hoagland [*57,58*] and Stephenson et al. [*131*].

That liver mitochondria can synthesize protein was first clearly indicated by the work of Simpson and colleagues [*61*]. From the autoradiographic results of Ficq [*39*] it appears likely that the nuclei of hepatic parenchyma cells, and even more so of Kupffer cells, may also synthesize protein.

CELL HETEROGENEITY WITHIN THE HEPATIC LOBULE

Early cytological studies demonstrated that intracellular structures showed marked quantitative differences among the parenchymatous cells within the lobule. Recent staining methods have shown such variation in almost every chemical substance and enzyme activity studied. (Fig. 27.) Direct biochemical measurements, by the method of "quantitative histochemistry," have become available recently [*129*]; these are valuable yardsticks for judging the staining observations [*86*].

Just as the distribution of the organelles within each parenchymatous cell is probably related to the direction of cytoplasmic and molecular movement within the cell, so the quantitative differences among adjacent cells probably reflect the direction of blood flow within the hepatic lobule. One of us [*84,86*] has tentatively suggested some functional meanings of such quantitative differences. The higher level of glucose-6-phosphatase activity in the peripheral cells may be related to the fact that these are the first cells to deposit glycogen. On the other hand, lipid is frequently formed first in the central cells, as are various hepatic pigments some of which are known to contain lipid. These central cells possess the highest levels of esterase and of β-hydroxybutyric dehydrogenase activities. The high succinic dehydrogenase and cytochrome oxidase activities of the peripheral cells, together with their numerous long mitochondria, suggest that in these cells which are exposed first to the blood entering the lobule oxidative respiration via the Krebs cycle may operate at a high level. In the cells around the central vein, which carries the blood leaving the lobule, there are fewer and more rounded mitochondria and the succinic dehydrogenase activity is lower. On the other hand, in these central vein cells glycolysis and other oxidative pathways may occur at a relatively high rate since their staining is higher than in other cells of the lobule for enzymes involved in these pathways, lactic dehydrogenase, DPNH- and TPNH-tetrazolium reductases, β-hydroxybutyric dehydrogenase and glutamic dehydrogenase. In the peripheral cells of the lobule acid phosphatase-rich lysosomes are larger and more numerous than in the more centrally located cells. This may reflect a higher rate of pinocytosis and other mechanisms by which proteins and other materials enter the cells from the nutrient-laden blood in the periphery.

Future studies will undoubtedly modify some of these interpretations, and the reader is urged to consider the uncertainties in these observations [*86*]. We are confident, however, that they will leave unshaken the importance of considering such observations in evaluating data from homogenates or subcellular fractions, particularly in altered physiological or pathological states in which there may be shifts in cell population as well as altered activity in different parts of the hepatic lobule. Similarly, metabolic heterogeneity must in some fashion be implicated in the differential susceptibility to toxic injury of cells in different parts of the lobule [*132,145*].

KUPFFER CELLS

The Kupffer cells are highly plastic cells which respond readily to stimulation. We have

studied their activation following partial hepatectomy, feeding of carcinogenic azo dyes, bile duct ligation and carbon tetrachloride poisoning. In all these situations the activated cells, like the few active Kupffer cells of the untreated animals, possess high acid phosphatase activity, localized in large lysosomes (*cf.* Figs. 6 and 7). Electron micrographs of such active Kupffer cells show a variety of complex bodies (Figs. 16 and 18) with the characteristics of lysosomes. These lysosomes, or "phagosomes" [*134*], frequently possess ferritin-like particles; they are surrounded by single membranes; and some are in the size range of other lysosomes. Similar phagosomes, with high acid phosphatase activity and single outer membranes but without electron-dense particles, have been described in erythrophagocytes [*35,91a*]. Schulz [*127*] has described the fine structure of a variety of "cytosomes" in the macrophages of human and rat lung which seem to us to be lysosomes (phagosomes) [*88*].

It is likely that the phagosomes are the periodic acid-Schiff positive bodies of Kupffer cells described by Atermann [*4*] and found to increase upon ethionine feeding of rats [*112,113*]. Whether or not they are also the sites of esterase activity [*139*] remains to be seen. As previously mentioned, the cell membrane in such active Kupffer cells shows high ATPase activity. Since in many cells such enzyme activity is found when the membrane is highly active [*85,87*], it seems likely that in the Kupffer cell the enzyme is somehow related to the prominent membrane flow which occurs in phagocytosis and pinocytosis. Our earlier finding [*95*] of high alkaline phosphatase activity in certain sinusoidal cells needs further study before associating it with Kupffer cells, let alone specific functions. This should be possible by incubating first for acid phosphatase then for alkaline phosphatase, using the lead method for one enzyme and one of the newer azo dye procedures for the other.

Enzymatic proteins, when injected intravenously into rats, induce the formation of numerous pinocytosis vacuoles in the Kupffer cells, and the proteins become concentrated in the lysosomes where they can be demonstrated by staining methods for their enzymatic activities [*96b*].

The most complete electron microscope study of phagocytosis in Kupffer cells is that of Hampton [*52*] who injected colloidal particles of thorotrast (thorium oxide) or mercuric sulfide intravenously into rats. Because of their high opacity to the electron beam, these particles can readily be followed [*106*]. The colloidal particles, perhaps covered with fibrin, quickly attach to the cell surface. Most particles are engulfed within vacuoles or small tubules formed from the cell surface. With time, the vacuoles increase in size (perhaps by fusion of small ones), number and density; the cell itself also increases in size. The phagocytized material remains in the Kupffer cell for a long time, perhaps indefinitely. There is no indication of transfer of material from Kupffer cells into parenchymatous cells. On the other hand, the latter are capable of phagocytosing the particles directly from the plasma (Disse space); they are apparently also able to transport colloidal material injected into the bile duct into the Disse space where it may then be phagocytized by the Kupffer cells.

Byers et al. [*16*] have combined histochemical staining methods for lipid with an interesting procedure for isolating Kupffer cells. They used a magnet to attract iron-laden cells, as Rous and Beard [*125*] had done to prepare Kupffer cells capable of division in tissue culture. Fine iron particles were injected into rats, the cells were loosened by perfusion, and forced through a fine silk screen. Separation from iron-free cells was achieved by differential centrifugation followed by repeated use of a strong magnetic field. In rats fed cholesterol and fat, stainable lipid droplets accumulate in the iron-containing cells. When cholesterol-4-C^{14} was used, the specific activity of the iron-containing cells was as much as four times greater, per unit weight of nitrogen, than the other liver cells. The authors conclude that the Kupffer cells are "important in the normal disposition of chylomicroanemic cholesterol." From experiments in which various colloidal particles and cholesterol in chylomicron form are injected together, the authors conclude that the Kupffer cells can be made to phagocytose particles rather than cholesterol.

As in the case of cholesterol, the Kupffer cells phagocytose fat from exogenous sources or tissue breakdown. These fat droplets contain considerable amounts of vitamin A [*114*]. Popper and Schaffner [*114*] discuss the role of Kupffer cells in blood pigment breakdown and bile pigment metabolism.

Wilson [*145*], in his plea for a more dynamic approach to the sinusoidal lining cells, considers them responsible for the formation of the argyrophilic reticulum which lies beneath them. The

virtual absence of fibroblasts from the reticulum network has recently been stressed by Harkness [*54*] and Popper [*112,113*]. Harkness points especially to the absence of fibroblasts among the cells growing from a liver explant in tissue culture, except for cells associated with bile duct epithelium. Popper leaves open the question whether the Kupffer cells form the reticulum "and thus assume the function of fibroblasts." He does, however, indicate that the Kupffer cells participate in the development of fibroblasts which follows injection of the moss polysaccharide, carrageenin. He believes that cholangioles and damaged liver cells may also make fibers, and that "dedifferentiation may be the common factor in stimulating the fibrosis."

Following feeding of 3′Me-DAB to rats, there is a marked increase in the argyrophilic fibers of the sinusoids. (Figs. 4 and 5.) Electron microscopy shows that the relation between Kupffer cells and fibers is as intimate as that usually seen with fibroblasts. (Fig. 16.) It should be noted that these are typical Kupffer cells, possessing the usual shape and features, such as phagosomes (Figs. 7 and 16); they are apparently not "undifferentiated."

As phagocytes, Kupffer cells of normal liver can concentrate antibodies but it is doubtful that they also manufacture them [*21*]. Gamma globulin is apparently not made in the normal liver [*77*]. Cohen et al. [*20*] have utilized the Coons fluorescent-antibody technic to study gamma globulin localization in livers of postnecrotic cirrhosis and various forms of hepatitis. They found gamma globulin in "reticuloendothelial cells showing transitions to plasma cells and exhibiting little or no phagocytosis of tissue breakdown products. These cells are assumed to form rather than engulf gamma globulin."

The extent to which Kupffer cells will phagocytose different bacterial species during perfusion of the isolated rat liver has been studied by Wardlaw and Howard [*141a*]. They conclude that "It seems likely that if all bacteria are equally effectively coated with opsonin, they would be treated in similar manner by the R.E.S. irrespective of basic differences in the organisms themselves."

The extensive work of Benacerraf, Halpern and co-workers [*6,7*] has shown that when small doses of colloidal particles are injected intravenously the Kupffer cells are able to clear nearly all the injected particles. These authors believe that the liver's blood supply assures its preeminent position as a filter of such particulate matter. They have ascertained the conditions under which the rate of clearance from the blood is a measure of Kupffer cell phagocytosis. The "phagocytic index" and other clearly defined units are quantitative expressions of such activity. Thus, when the Kupffer cells are injured by endotoxin, their phagocytic index and "critical R.E. dose" fall; or when treatment with the yeast extract, zymosan, renders the mice more resistant to infection, these indices rise.

Such quantitative measurements of Kupffer cell metabolism could readily be combined with staining procedures, including those for the acid phosphatase of lysosomes, ATPase of the plasma membrane, and perhaps oxidative enzymes and esterase. They could also be correlated with electron microscopy. Such integrated studies might, for example, help in the understanding of fundamental aspects of phagocytosis which have been described quantitatively, such as the preferential phagocytosis of one colloid over another.

Attention should be drawn to the work of Wattiaux et al. [*142*] who modified the technic, already outlined, for the separation of Kupffer cells from parenchymal cells, and analyzed the cells for five lysosomal enzymes. All five were present in the Kupffer cells, three with higher specific activities (activity per unit of nitrogen) and two with somewhat lower ones. The observed values may well be less than those of the original cells, because of the iron uptake. Straus [*133*] found that when protein is absorbed by kidney lysosomes (droplets) appreciable solubilization of the lysosome enzymes occurs. de Duve [*26*] offers an alternative explanation for Straus's observation, that the enlarged lysosomes may be unusually fragile and more readily destroyed in the course of isolation.

The unusually high rate of nucleic acid and protein metabolism in Kupffer cells has recently been studied by autoradiography [*39*].

The newer technics of biochemical cytology should help settle more definitively many questions of long standing. One of the oldest of these is whether all sinusoidal cells, the flat "lining cells" and larger active Kupffer cells, are all "one cell type in various phases of activity" [*4,32,104,126a*]. An affirmative answer seems required to explain the rapid increase in lysosome number and in size and enlargement of all lining cells when rats are fed 3′Me-DAB [*89*] or

are injected with proteins [*96b*]. In the latter case this occurs in a few minutes, too quickly to make mobilization from elsewhere in the body [*145*] likely.

SPECIES DIFFERENCES

Species differences in hepatic biochemical cytology are to be anticipated, as counterparts of the many functional differences reported in the literature. Thus the normal rat does not deposit lipofuscin in its cells as does man. As noted early by Gomori [*48*], the sinusoid wall in the rat shows no staining when incubated for alkaline phosphatase activity whereas in man its staining intensity exceeds that of the bile canaliculi [*84*]. In rat and man the bile canaliculi stain when incubated with adenosine-5′-monophosphate at pH 7.2, but not in the rabbit [*137*]. Species differences in the form of mitochondria in different parts of the lobule and their response to fasting were described in 1933 by Kater [*62*].

CONCLUSION

We hope our presentation has indicated the kinds of questions currently being asked regarding hepatic cells and some of the technics by which answers may be found. With the increased availability of fresh tissue by the technic of needle aspiration biopsy, electron microscopic studies on human material may be expected to increase rapidly. Cytochemical staining procedures can best be carried out on such freshly-fixed specimens, but postmortem material also is useful, even for demonstrating enzyme activity.* Aside from the functional information that such enzyme preparations provide,† we have seen that they are useful in delineating cell structures (ATPase for bile canaliculus microvilli, 5′-nucleotidase for sinusoid microvilli, acid phosphatase for phagocytosis vacuoles) and cell types (alkaline phosphatase for cholangioles [*146*], and acid phosphatase for Kupffer cells, degenerating foci and regenerating nodules). It is safe to predict that such staining technics will reveal cell changes in situations like early biliary obstruction in which, as Cameron [*17*] describes, by conventional technics "the liver cells are singularly little affected."

Rouiller [*124*] suggests that the electron microscope now permits the study of early degenerative changes while the cell may still look normal with light microscope, and degeneration of cell organelles can be studied even when cell death does not occur. Palade [*100*] points to the value of the electron microscope in tracing the transformation of these organelles in varying functional states.

Physiological and pathological alterations in biochemistry and morphology will increasingly be sought inside the cell organelles and within the absorptive and excretory surfaces of the cells. As we have seen, some hepatic malfunctions already are attributed to defects in the enzymatic machinery of the ergastoplasm. Others may involve deficient activity of the absorptive or excretory surfaces. For example, Smetana and Johnson [*130*] and Ehrlich and Ratner [*31b*] have reported cases of hepatitis in the neonatal period in which, by the conventional light microscopic technics, bile canaliculi appear to be absent. Schaffner and Popper [*126b*] have proposed that the primary site of injury in intrahepatic cholestasis is the parenchymal cell membrane within the bile canaliculus. Popper [*112,113*] suggests that the functional significance of hepatic fibrosis is the interference of exchange between blood and hepatic cells by interposition of material at the base of the lining cells.

With these newer technics it may be possible better to pinpoint which biochemical-cytological lesions are reflected in certain of the clinical tests of liver function.

Slowly the foundation for a macromolecular cell pathology is being built.

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* The acid phosphatase preparation shown in Figure 12 is of human liver not placed in fixative until twenty-four hours after death. The same liver was adequate for localization of the other enzymes studied: ATPase, 5′-nucleotidase, alkaline phosphatase and DPNH-tetrazolium reductase.

† For their utilization in studies of structure-function relations in the cells of kidney, tumors and brain see [*91b*], [*91c*] and [*5b*], respectively.

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Renal Medullary Necrosis*

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RENAL medullary necrosis is defined as an ischemic necrosis involving the renal medulla which, in the absence of localizing obstructive or vascular factors, is generally bilateral and most often is associated with infections of the urinary tract. Diabetic patients and patients with obstructive uropathy are predisposed to medullary necrosis only insofar as their primary underlying disease process predisposes them to infection, the prime etiological factor in the genesis of this variant of pyelonephritis.

The necrosis is anatomically limited to the renal medulla, hence the term "renal medullary necrosis" is preferable to such terms as necrotizing papillitis, papillary necrosis or necrotizing pyelonephritis, even though the initiating factors, such as pyelonephritis, may be present throughout the cortex.

The clinical course is characterized by lumbar pain, hematuria, pyuria, bacteriuria and sepsis; occasionally also by the passage of whole renal papillae or sections thereof in the urine. The onset may be abrupt with a fulminating acute renal failure, or more insidious when there is an underlying disease state such as an obstruction of the urinary tract; in the latter instance the diagnosis may be delayed until progressive involvement results in azotemia and uremia associated with sepsis. This disease entity is not rare, as was once thought.

The present study includes six proven cases. One, demonstrated by renal biopsy, suggests that the disease process may be reversible.

CASE HISTORIES

CASE 1. J. A., a fifty year old man, was transferred to Georgetown University Hospital because of anuria of four days' duration. He had been in good health until three weeks before admission when he noted the onset of pain in the right side of the flank. Five days before admission he awoke with bilateral moderately severe pain in the flank, on the right side radiating into the right testicle. He was admitted to another hospital where a plain roentgenogram of the abdomen suggested the possibility of a calculus in the lower third of the right ureter. Intravenous pyelography showed poor filling of the right pelvis and ureter, and a filling defect in the lower third of the right ureter. Three days before admission cystoscopy and retrograde pyelography revealed chronic cystitis. Cultures from both renal pelves were sterile. Total anuria followed cystoscopy. Two days before admission an attempt to pass a catheter into the bladder was unsuccessful. A low grade fever developed, with marked rigidity of the lower abdomen. A suprapubic cystostomy revealed the bladder to be devoid of urine. On the day of transfer his serum urea nitrogen was 80 mg. per 100 ml., serum sodium 129 mEq. per L., potassium 5.2 mEq. per L., chloride 106 mEq. per L., carbon dioxide combining power 24 mEq. per L.

On physical examination the temperature (rectal) was 101.6°F., pulse 90 per minute, respirations 24 per minute, and blood pressure 180/110 mm. Hg. He was confused and disoriented, having visual hallucinations. Dullness to percussion, decreased breath sounds and fine dry rales were noted at the right base of the lung posteriorly. There was tenderness to percussion over the right costovertebral angle. The prostate was symmetrically enlarged and firm. There were no pathologic reflexes.

Significant laboratory findings included microscopic hematuria and pyuria, a blood hematocrit of 42 per cent, a white cell count of 15,050 per cu. mm. with 91 per cent segmented neutrophils, 4 per cent lymphocytes and 5 per cent monocytes, an erythrocyte sedimentation rate (corrected) of 42 mm. per hour, a total eosinophil count of 9 per cu. mm., serum urea nitrogen of 116 mg. per 100 ml., creatinine of 14.7 per 100 ml., uric acid 18.3 mg. per 100 ml., phosphorus 6.7 mg. per 100 ml., serum sodium 12 mEq. per L., potassium 5.4 mEq. per L., chloride 90 mEq. per L. A roentgenogram of the chest showed slight elevation of the right leaf of the diaphragm and several areas of increased density in the left lung field. An electrocardiogram showed a sinus tachycardia with a rate of 110 per minute, S-T segment depression in leads 1,

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FIG. 1A. Case I. Section through medulla of kidney. Note congested and ruptured blood vessels, interstitial edema, increase in fibrosis and diffuse interstitial inflammatory reaction. Hematoxylin and eosin × 109.



FIG. 1B. Case I. Higher magnification of a small portion of Figure 1A. Note congested capillaries and massive interstitial edema. Hematoxylin and eosin × 500.

aVL and V_1 through V_5. One culture of the urine and two cultures of the blood showed no growth.

Therapy consisted of restriction of protein, potassium and intake of fluid. He continued to vomit persistently; he was confused, disoriented, and had visual hallucinations. The serum urea nitrogen rose to 131 mg. per 100 ml., with a serum potassium of 5.1 mEq. per L. On the third day pulmonary edema suddenly developed which failed to respond to routine measures. Within a few minutes tonic-clonic convulsions developed. An electrocardiogram showed a change from sinus tachycardia to ventricular fibrillation. He died shortly thereafter.

Autopsy revealed pulmonary congestion and edema, healed tuberculous nodules with one caseous mass in the upper left lobe of the lung, bronchopneumonia of the upper left lobe of the lung, passive congestion of the liver, right ureteral calculus, moderate dilatation of the ureters, pyelonephritis and bilateral renal medullary necrosis. (Figs. 1A and 1B.)

Comment: This patient illustrates the fulminating course which may characterize this disease entity. He was an active healthy man, playing golf when the first discomfort in the flank occurred, and died within a week as a result of ventricular arrhythmia presumably related to the bacteremia developing from medullary necrosis. Obstruction and instrumentation of the urinary tract were prominent factors in pathogenesis.

CASE II. H. H., a sixty year old white man, was admitted to Georgetown University Hospital because of anuria of four days' duration. The past history included recurrent sore throats during childhood, hypertension and mild anginal syndrome for several years, a heart murmur, trauma to the right knee with persistent arthralgia, and two painful episodes diagnosed as renal colic.

Twelve days before admission he was exposed to calcium cyanide spray inside his barn, followed by painless hematuria of three days' duration. He was admitted to another hospital where the serum urea nitrogen was 20 mg. per 100 ml., later rising to 191 mg. per 100 ml., the serum potassium increasing to 6.9 mEq. per L. Intravenous urography was performed with 50 per cent Hyopaque.® Immediately following the dye injection the patient became flushed, nauseated, and then vomited. These symp-

TABLE I (CASE I)

	Serum Urea Nitrogen	Creatinine	Uric Acid	Phosphorus	Sodium	Potassium	Chloride	Osmolarity (mOsm./L.)	Weight (lb.)
	(mg./100 ml.)				(mEq./L.)				
Before dialysis	219	25	27	15	136	7.5	89	367	251
After dialysis	86	12	16	8.6	140	4.5	101	344	...

toms subsided spontaneously in thirty minutes. On the next day cystoscopy and bilateral pyelography were performed under intravenous Pentothal® anesthesia, with essentially normal findings. Fever developed (averaging 101°F.) for the next forty-eight hours, transient bilateral lumbar pains, and oliguria. Four days before admission he had anuria and became nauseated, with general malaise and vomiting.

On physical examination at transfer the temperature (oral) was 99.0°F., blood pressure 205/100 mm. Hg, pulse 104. There were visible pulsations of the neck veins, cardiomegaly was present with a harsh grade 4 apical systolic murmur, and left costovertebral angle tenderness and a distended tender abdomen with diminished bowel sounds and tympany were noted.

The significant laboratory findings included hematuria, pyuria, cylindruria, a blood hematocrit of 38 per cent, a white cell count of 8,350 per cu. mm. with 52 per cent segmented and 29 per cent band neutrophils, an erythrocyte sedimentation rate (corrected) of 42 mm. per hour. The serum urea nitrogen was 170 mg. per 100 ml., the serum sodium was 138 mEq. per L., potassium 7.0 mEq. per L., and chloride 96 mEq. per L. The platelet count was 137,000 per cu. mm. The serum creatinine was 19.6 mg. per 100 ml., the phosphorus 15.5 mg. per 100 ml., the uric acid 25 mg. per 100 ml., and the blood sugar 126 mg. per 100 ml. Throat culture grew 2 plus beta-hemolytic streptococcus. Blood and urine cultures showed no growth. The antistreptolysin titer was 1:160. The lupus preparation was negative. An electrocardiogram revealed high peaked T waves consistent with hyperkalemia and occasional premature ventricular contractions.

The day after admission bilateral conjunctival hemorrhages were noted. The azotemia increased to 219 mg. per 100 ml., and the hyperkalemia to 7.5 mEq. per L. At this point hemodialysis was carried out with the chemical changes taking place shown in Table I. He had anuria for the first three hospital days. On the fourth hospital day his output of urine was 1,150 ml. This increased to 2,920 ml. by the sixth hospital day, but fell to 1,600 ml. on the eighth hospital day. His serum urea nitrogen continued to rise, reaching 238 mg. per 100 ml. on the eighth day. Because of the declining urinary output, cystoscopy and ureteral catheterization were carried out and revealed bilateral ureteral obstruction by blood clots. A left pyelostomy was performed because of inability to maintain adequate drainage via the ureteral catheters. During the procedure shock developed and the patient died.

Autopsy findings disclosed pulmonary edema, focal atelectasis, pulmonary thrombosis, left ventricular hypertrophy, dilatation of the mitral valve, coronary atherosclerosis, myocarditis, bilateral ureteral dilatation, acute necrotizing ureteritis, and acute pyelonephritis with medullary necrosis, bilaterally. (Fig. 2.)

Comment: The fever, pain and oliguria suggested necrotizing papillitis occurring after retrograde pyelography in a man previously allergic to the intravenous administration of iodide. The pre-existing hematuria was probably due to an acute pyelonephritis. The role of the chemical spray could not be assessed. The case illustrates the common association of infection and instrumentation in the pathogenesis of this variant of acute renal insufficiency.

CASE III. A. W., a seventy year old woman, was transferred to Georgetown University Hospital because of anuria of three days' duration. Five years before hospital admission she noted polyuria, polydipsia and gradual weight loss. Three years later exertional dyspnea, orthopnea and nocturia developed. Ten days before admission she began to complain of malaise and weakness. Four days before admission she was digitalized because of marked shortness of breath. At this time she passed only scant amounts of bloody urine for a twenty-four-hour period. Her blood pressure was 90/60 mm. Hg. Right costovertebral angle tenderness developed, and she had complete anuria during the next seventy-two hours. Her blood sugar was found to be 468 mg. per 100 ml. The serum urea nitrogen was 64, rising to 106 mg. per 100 ml. before transfer.

Physical examination revealed a pale, tachypneic



FIG. 2. Case II. Section through medulla of kidney. Inflammatory reaction has produced dissolution of tubules in upper half of section. A glomerulitis is also present. Hematoxylin and eosin × 70.

elderly woman who vomited frequently. The temperature was 98.6°F., pulse 90, respiration 36 and blood pressure 188/82 mm. Hg. She had cardiomegaly, a bounding pulse, apical and aortic systolic murmurs, moist basilar rales, ascites and dependent edema.

Laboratory examination revealed a hematocrit of 34 per cent, a white cell count of 23,400 per cu. mm. with 64 per cent segmented and 28 per cent band neutrophils. The erythrocyte sedimentation rate (corrected) was 34 mm. per hour. The total eosinophil count was 12 per cu. mm. The serum urea nitrogen was 125 mg. per 100 ml., fasting blood sugar 320 mg. per 100 ml., uric acid 13.2 mg. per 100 ml., and the creatinine 13.2 mg. per 100 ml. The serum sodium was 120 mEq. per L., potassium 5.2 mEq. per L. and chloride 91 mEq. per L. Escherichia coli grew on cultures of the blood.

The patient had anuria for the first ten hospital days, after which her output of urine increased somewhat but remained between 500 and 2,000 ml. per 24 hours. Hemodialysis was performed on the third, eleventh and twenty-ninth hospital days to control the symptoms of uremia. The chemical changes are shown in Table II.

Cystoscopy with retrograde urography was performed on the fifth and ninth days. On both occasions the bladder was found to be very contracted. The right ureteral orifice could not be identified. Retrograde urography of the left ureter and pelvis showed a

TABLE II (CASE III)

	Serum Urea Nitrogen	Creatinine	Uric Acid	Phosphorus	Sodium	Potassium	Chloride	Osmolarity (mOsm./L.)	Weight (lb.)
	(mg./100 ml.)				(mEq./L.)				
1, Before dialysis	174	19	18	6.1	120	6.0	91	397	202
After dialysis	60	11	3.8	3.5	137	5.0	102	314	199
2, Before dialysis	230	31	25	22.5	136	8.1	103	380	188
After dialysis	63	11	8	7.7	143	5.5	07	338	193
3, Before dialysis	135	19	14	9.1	117	4.0	181	332	178
After dialysis	47	7.7	7	4.2	134	4.0	106	308	183

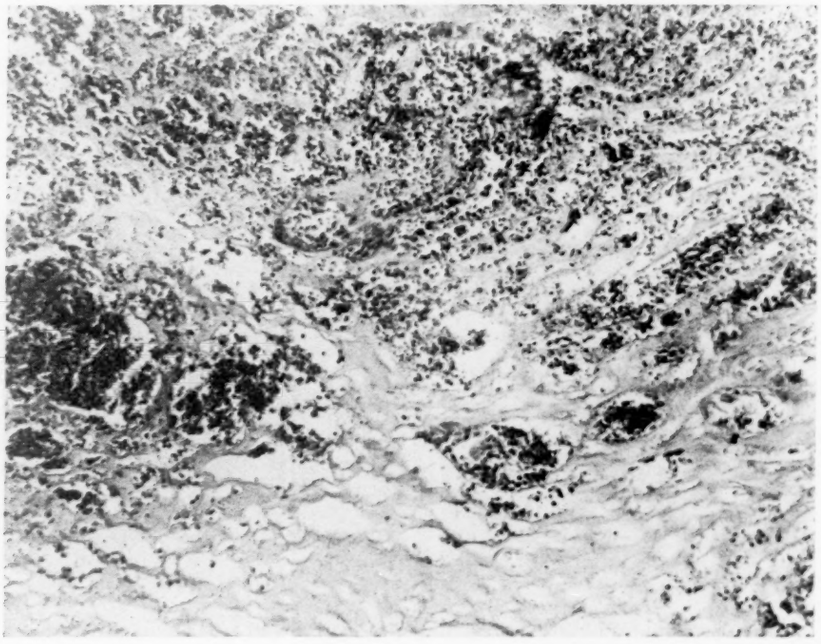


FIG. 3. CASE III. Dense inflammatory reaction at edges of necrotic area of right kidney medulla. Hematoxylin and eosin × 70.

walled-off pyonephrosis. The right ureter was successfully catheterized on the twenty-fifth hospital day and urography revealed hydronephrosis on that side. Her hospital course was complicated by multiple infections with two episodes of septicemia which responded to antibiotic therapy. Marked pyuria was present, with changing bacterial flora throughout her course. Terminally her course was characterized by a decreasing urinary output with uremia and congestive heart failure which failed to respond to therapy. Death occurred in congestive failure.

Autopsy findings disclosed bilateral pulmonary edema and atelectasis, cerebral, coronary and aortic atherosclerosis, left ventricular hypertrophy and dilatation, uremic myocarditis, myocardial fibrosis, chronic cystitis, bilateral hydroureters, left pyonephrosis with marked parenchymal atrophy, benign nephrosclerosis and right medullary necrosis and chronic pyelonephritis. (Fig. 3.)

Comment: This patient illustrates another frequent combination in the pathogenesis of this disease, *viz.*, age, diabetes, infection and instrumentation. It is curious to note that although both sides were instrumented separately, papillitis was a unilateral development, on the right side.

CASE IV. C. M., a forty-five year old woman, was transferred to Georgetown University Hospital by the police. She had been found on a park bench in a debilitated condition, covered with feces, and suffering from exposure. She had been admitted to another hospital where she was noted to be hypotensive and to have oliguria. Blood chemical studies included a serum urea nitrogen of 280 mg. per 100 ml. and fasting blood sugar of 300 mg. per 100 ml. Blood and urine cultures grew an E. coli sensitive to tetracycline. The past history included chronic alcoholism, weakness of both legs for three months and diarrhea for two weeks' duration.

Physical examination revealed a temperature of 99°F. by rectum, pulse 100, respiration 22 and blood pressure 120/70 mm. Hg. There was a widespread erythematous pigmented skin eruption. Many of the lesions were circinate, some showed purpuric areas, others were telangiectatic. The lips were covered with clotted blood and white plaques were noted on the buccal mucosa. There was a grade 2 systolic murmur at the apex. Hepatomegaly was present. A polypoid mass was noted on the left posterolateral wall of the rectum. A trace of pitting pretibial edema was present.

Urinalyses revealed isosthenuria and 3 to 4 plus proteinuria, with many glitter cells, red blood cells, oval fat bodies and bacteria in the sediment. The hematocrit was 24 per cent, hemoglobin 7 gm. per 100 ml., the white cell count was 18,000 per cu. mm. with 81 per cent segmented and 12 per cent band neutrophils, 2 per cent lymphocytes and 5 per cent eosinophils. The platelet count was 41,000 per cu. mm. and the total eosinophil count was 144 per cu. mm. The serum urea nitrogen was 196 mg. per 100 ml., the fasting blood sugar was 280 mg. per 100 ml. and the plasma acetone was negative. The serum sodium was

TABLE III (CASE IV)

	Serum Urea Nitrogen	Creatinine	Uric Acid	Phosphorus	Sodium	Potassium	Chloride	Osmolarity (mOsm./L.)	Weight (lb.)
	(mg./100 ml.)				(mEq./L.)				
Before dialysis	300	29	28	8.8	120	3.6	92	353	...
After dialysis	85	8	11	2.7	140	3.7	108	321	...

TABLE IV (CASE IV)

	Serum Urea Nitrogen	Creatinine	Uric Acid	Phosphorus	Sodium	Potassium	Chloride	Osmolarity (mOsm./L.)	Weight (lb.)
	(mg./100 ml.)				(mEq./L.)				
Before dialysis	240	25	28	...	150	6.5	110	...	...
After dialysis	50	7	14	7	145	4.0	106	...	...

119 mEq. per L., potassium 4.5 mEq. per L., chloride 78.5 mEq. per L. and the carbon dioxide combining power 10.9 mEq. per L. A blood culture grew E. coli. A roentgenogram of the chest showed bronchopneumonia in the right lower lung. An electrocardiogram revealed a tachycardia of 107 per minute and a prolonged Q-T interval.

During the thirty-six hours following admission she continued to have oliguria, the uremia became progressively worse; she began to have intermittent low grade fever and the hyponatremia remained. On the third hospital day hemodialysis effected the chemical changes shown in Table III.

During the following four days hypotension developed with warm, dry skin, increasing azotemia (serum urea nitrogen 240 mg. per 100 ml.), hyperglycemia (432 mg. per 100 ml.) and hyperkalemia (7.0 mEq. per L.), with corresponding changes in the electrocardiogram. Hemodialysis was carried out again, with the chemical changes shown in Table IV.

Although she improved clinically, she became increasingly febrile and dependent on vasopressor agents. A blood culture obtained at this time grew E. coli and a urine culture grew a coagulase-positive hemolytic staphylococcus and Aerobacter aerogenes. She died in irreversible shock on the tenth hospital day.

Autopsy revealed pulmonary edema, hypostatic pneumonia, severe fatty infiltration of the liver with early portal cirrhosis, petechial gastric hemorrhage, septic degeneration of the adrenal cortex, perinephric and renal cortical abscesses, bilateral renal medullary necrosis and suppurative pyelitis. (Figs. 4A and 4B.)

Comment: Malnutrition, exposure and fecal contamination probably contributed to an E. coli pyelonephritis in this patient. The infection could not be controlled. The kidney showed medullary necrosis. Constitutionally, the patient had the typical course of a gram-negative septicemia with shock.

CASE V. E. F., a sixty-one year old Negro woman who had diabetes for three years, was admitted because of pain and swelling of the left lower extremity. Two years prior the right metatarsal had been amputated for diabetic gangrene. Her diabetes had been controlled by diet alone. Testing of the urine for sugar had been haphazard.

Physical examination revealed a temperature of 101.8°F., pulse 90 per minute, and a blood pressure of 170/80 mm. Hg. An infected ulcer, 1.5 cm. in diameter, on the sole of the left foot had produced a marked cellulitis of the entire extremity. A grade 3 aortic systolic murmur was present. Bilateral lenticular opacities were noted. The significant laboratory data included microscopic pyuria, a hemoglobin of 11.5 gm. per cent, a blood sugar of 108 mg. per 100 ml. and a serum urea nitrogen of 18 mg. per 100 ml. X-ray film revealed tortuous calcified vessels in the legs, left ventricular enlargement and calcification of the aortic knob. Culture of the ulcer grew Proteus

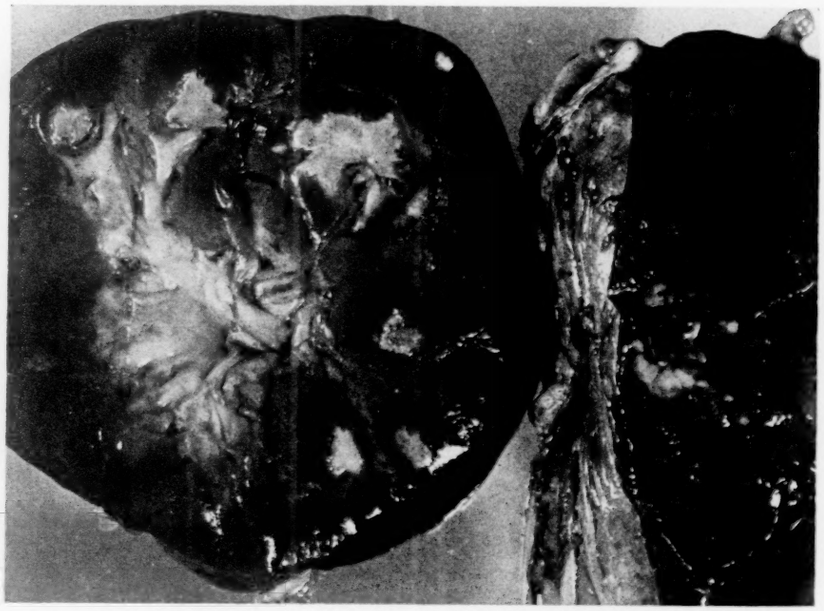


FIG. 4A. Case IV. Gross view of necrotic papillae.

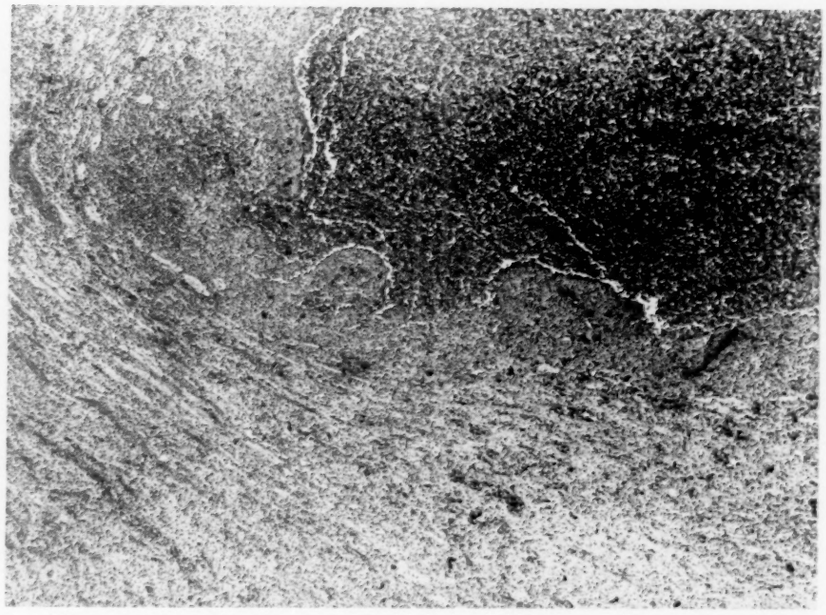


FIG. 4B. Case IV. Low power section through papillae. Note the dense reaction, the margin of necrosis and the beginning separation of the purulent slough. The area in the upper right corresponds to the white papilla tips seen in 4A. Hematoxylin and eosin × 28.

aerogenes which was sensitive to Chloromycetin® and novobiocin. Administration of the latter drug was begun. Transection of the fourth and fifth metatarsal bones was carried out under Pontocaine® spinal anesthesia. The patient was given 1,500 cc. of 5 per cent glucose in water without insulin during the operation and also 5 mg. Aramine® administered intramuscularly, for transient hypotension. Following administration of the latter drug the blood pressure rose sharply to 240/120 mm. Hg and then gradually returned to the admission level. After the operation the patient appeared lethargic, disoriented, and experienced a frank chill with subsequent rise in temperature. An erratic temperature curve was characteristic of the remainder of her hospital stay. The patient was catheterized to insure accurate urine out-

put records and the urine output was found to be adequate. Two days later the patient began to vomit. The hemoglobin fell to 9 gm. per cent. She was given 500 cc. of stored whole bank blood. Pinch grafting was carried out. One week later the urine was noted to contain innumerable white cells, with 2 plus albuminuria. Gantrisin® therapy was begun. The urine culture grew E. coli, sensitive to Furadantin® and resistant to all other antibiotics. An indwelling catheter was reinserted. Two days later there was frank epistaxis. The serum urea nitrogen was 78 mg. per 100 ml. Oliguria developed. At this point the diagnosis of renal medullary necrosis was entertained. Electrocardiogram at this time revealed tall peaked T waves in all leads. The urea nitrogen was 115 mg. per 100 ml., the creatinine 5.6 mg. per 100 ml. The sodium was 136 mEq. per L., potassium 8.3 mEq. per L., chloride 95 mEq. per L. and carbon dioxide content 10 mEq. per L. Cystoscopy and retrograde examination revealed no obstruction. Peritoneal lavage and the administration of cation exchange resins was carried out for a continuous forty-four-hour period, with an exchange of 35,000 cc. of fluid through the peritoneal cavity. At the conclusion of the dialysis the serum urea nitrogen was 109 mg. per 100 ml., sodium 130 mEq. per L., potassium 4.3 mEq. per L., chloride 80 mEq. per L. and carbon dioxide combining power 25.8 mEq. per L. The urine output on consecutive days was 150, 100, 150, 170, 170, 185 and 132 ml. The patient gained six pounds during the dialysis. Clinically, there was only transient improvement in the patient's condition. Two days following the dialysis she suddenly had a convulsion and died on the sixty-fourth hospital day. Autopsy revealed acute and chronic pyelonephritis, moderate nephrosclerosis, diabetic intercapillary glomerulosclerosis and an extensive gross bilateral renal medullary necrosis with involvement of all papillae.

Comment: The potential precipitating factors included renal ischemia due to a hypotensive episode with spinal anesthesia, the administration of Aramine followed by an acute rise in blood pressure, blood transfusions, sulfa drug sensitivity and infection. Clinically, the sudden deterioration of this elderly diabetic patient following surgical intervention suggests a gram-negative bacteremia with resultant acute pyelonephritis superimposed on kidneys already compromised by chronic pyelonephritis and diabetic glomerulosclerosis; the end result was acute bilateral medullary necrosis.

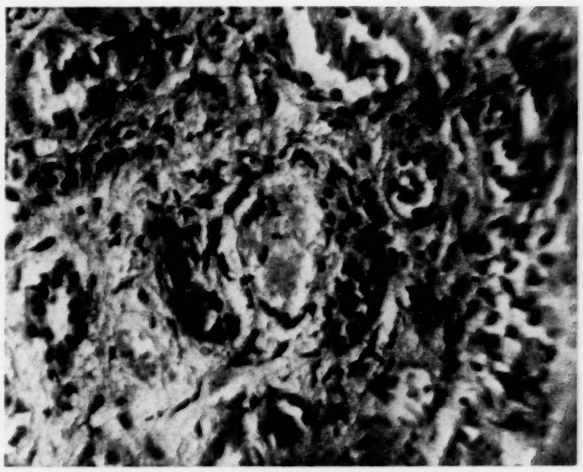


FIG. 5. Case VI. Section through medullary portion of biopsy specimen of patient. Note interstitial edema, congestion of blood vessels and necrosis of tubules. Hematoxylin and eosin × 200.

CASE VI. S. W., a fifty-nine year old white man, was admitted because of lumbar pain and anuria of eighteen hours' duration. In the past he had had a herniorrhaphy (thirty-five years ago), appendectomy (thirty years ago), hemorrhoidectomy (two years ago), gonorrhea (twenty-one years ago) treated with irrigations and followed by arthritis, and recurrent hydrocele. There was a family history of diabetes but the patient had had regular medical examinations, including blood sugar determinations, without any indication of diabetes.

Two months previously he had experienced urgency, frequency and urinary tenesmus lasting three days. Two weeks before he had left inguinal pain and subsequently a suprapubic ache, anorexia, sharp lumbar pain and then asymptomatic anuria. After sixteen hours of total anuria, he consulted a physician who inserted a catheter but found no urine. He was admitted to the hospital with an indwelling catheter.

Physical examination revealed an obese man with mild hypertension (160/100 mm. Hg), low grade fever, and slight tenderness in the right flank. Retrograde pyelogram showed a serrated bladder, normal ureters and calyces. The urine had a specific gravity of 1.003, with 2 plus albuminuria, no sugar. Microscopic examination revealed 1 to 3 red cells, more than 50 white cells, numerous small and large clumps, 30 per cent glitter cells, oval fat bodies, several large mononuclear macrophages, bladder epithelial cells, hyaline and granular casts. Phase contrast microscopy revealed chiefly two types of bacteria, a motile long rod and a bipolar rod. He had anemia (11.9 gm. per cent hemoglobin), mild azotemia (urea nitrogen 32 mg. per 100 ml.), and acidosis (CO_2 19 mM./L.). The urine culture grew E. coli intermedium. He was treated promptly with antibiotics. He voided 460, 1,680 and 2,400 ml. on the first three days. The fever and pain subsided and the urine output continued to increase, although the serum urea nitrogen rose successively to 108 mg. per 100 ml. on the seventh hospital day. Percutaneous renal biopsy was performed

TABLE V

RENAL MEDULLARY NECROSIS

(SUMMARY OF CLINICAL FEATURES IN SIX PATIENTS)

Case No. and Patient's Initials	Age (yr.) and Sex	Diabetic History	Instrumentation	Urinary Obstruction	Oliguria or Anuria	Pain in the Flank or Abdominal Pain	Chills and/or Fever	Roentgenographic Findings	Tissue			Medullary Necrosis
									Pyelonephritis	Kimmelstiel-Wilson's Disease	Nephrosclerosis	
I, J. A.	50, M	No	Yes	Yes	Yes	Yes	Fever	Poor filling; stone	Yes, culture negative	No	No abnormalities	Bilateral
II, H. H.	60, M	No	Yes	No	Yes	Yes	Fever	Normal intravenous and retrograde pyelogram	Yes, culture negative	No	Yes	Bilateral
III, A. W.	70, F	Yes	Yes	Probably	Yes	Yes	Chills and fever	Hydronephrosis	Yes, E. coli	No	Yes	Unilateral
IV, C. M.	45, F	Possibly	No	No	Yes	No	Fever	None	Yes, E. coli	No	Yes	Bilateral
V, E. F.	61, F	Yes	Yes	No	Yes	Yes	Yes	No obstruction	Yes, E. coli	Yes	Yes	Bilateral
VI, S. W.	59, M	No	No	No	Yes	Yes	Yes	Non-functioning intravenous pyelogram	Yes, E. intermedium	No	Yes	Unknown

on the tenth hospital day. (Fig. 5.) The glomeruli were normal except for some lymphocytic infiltration in the periglomerular lymphatics. In the cortex there were areas of infiltration with polymorphonuclear and mononuclear cells and an increase in interstitial fibrosis. In the medulla some tubules showed epithelial cell hypoplasia. Noted were severe edema and compression of tubules, with one cyst. The stroma was infiltrated with polymorphonuclears and small mononuclear cells. Periodic acid-Schiff stain showed heavy deposits of fibrin-like material in medullary areas. The biopsy specimen was thought compatible with moderately severe chronic pyelonephritis and a recent process in the papilla best described as papillitis or early medullary necrosis. Some tubules had undergone ischemic changes, others were in a reparative phase. The patient made an uneventful recovery and was discharged on the sixteenth hospital day.

He died two years later of prostatic carcinoma. The kidney showed only nephrosclerosis and the scars of a healed pyelonephritis.

Comment: A type of medullary pyelonephritis is represented in this case in which inflammatory edema of the papilla can obstruct collecting ducts and totally suppress the flow of urine. The etiology of the anuria would have been completely obscure without the negative findings in the retrograde films and the positive findings in the papilla on biopsy. The inflammatory process apparently did not progress to frank medullary necrosis.

The major features of these six patients are summarized in Table V.

REVIEW OF THE LITERATURE

Von Friedreich in 1877 [*1*] first described the pathological findings of medullary necrosis. This was followed a decade later by a series of three cases in the English literature by Turner [*2–4*], thus establishing this disease as a distinct clinicopathologic entity. It is of interest that in these initial cases reports the association of medullary necrosis with obstructive uropathy and sepsis, its occurrence in both sexes and in diabetic and non-diabetic patients were demonstrated. Turner, noting the association of medullary necrosis with rheumatic endocarditis, proposed vascular thrombosis as the inciting cause. Chiari in 1899 [*5*] suggested that mechanical twisting of the papillae secondary to increased intrapelvic pressure due to obstruction of the urinary tract might be an etiologic factor. The same year Stoudensky [*6*] reported on six patients with renal medullary necrosis, five of whom had unilateral involvement; in all patients the condition was associated with obstruction and hydronephrosis. A quarter of a century

passed before interest in this clinical entity was reawakened by a case reported by Artusi [*7*], in which neither diabetes nor obstruction was present. Schomer [*8*] returned to the earlier concept of Turner, that a vascular lesion was responsible for this disease, and he proposed that capillary thrombosis engendered by septicemia (as noted in his three cases) was the inciting factor. The possibility that renal parenchymal infection with associated impairment of the renal vasculature predisposes to medullary necrosis was recorded by Grauhan in 1934 [*9*].

In France, Foulon [*10*] reported a case of unilateral medullary necrosis after retrograde pyelography and demonstrated that the necrosis may be iatrogenic presumably by increased intrapelvic pressure from or sensitivity to the injected contrast media in the renal pelvis. In separate communications in 1937 Froebese [*11*] and Gunther [*12*] established the now well recognized association of diabetes mellitus with infection of the urinary tract and medullary necrosis. Gunther [*12*] found diabetes mellitus to be present in eight of his ten cases, and suggested that bacterial toxins may cause the necrosis indirectly by a vasoparalytic effect on the blood vessels which supply the renal pyramids, resulting in ischemic infarction. By 1948 Gunther [*13*] personally had observed twenty-nine patients with medullary necrosis and had found diabetes in twenty-five. He also described radiological features suggestive of this disease in the pyelograms of these patients.

In 1942 Harrison and Bailey [*14*] presented data on three diabetic patients with medullary necrosis, which they termed a "necrotizing pyelonephritis." In 1946 Robbins, Mallory and Kinney [*15*] reported twenty-six cases, largely from the Boston City Hospital. They considered medullary necrosis in the diabetic patient to be a form of acute pyelonephritis with a predilection for the renal pyramids. The initial lesion was depicted as a coalescence of abscesses in the outer zone of the pyramids, ischemia and necrosis of the medulla ultimately occurring as a result of compression of the arterial capillaries leading to the pyramids, by the inflammatory exudates. They pointed out that in non-diabetic patients with obstruction of the urinary tract the increased intrapelvic pressure, by causing dilatation of the tubules and compression of the interstitial tissue could compromise the pyramidal blood supply and in this way initiate medullary necrosis.

In the following year Edmondson, Martin and Evans [*16*] suggested that medullary necrosis in patients with diabetes was, in a sense, a form of diabetic gangrene, that is, necrosis with little inflammatory response. They noted also that sclerosis of the smaller renal vessels, which occurs more commonly in diabetic subjects, coupled with intercapillary glomerulosclerosis so frequently present in these patients, was just as important in compromising the pyramidal blood supply as were the inflammatory exudates referred to earlier.

In 1949 Robbins and Angrist [*17*] pointed out that abnormal fat metabolism may play a part in the pathogenesis of this disease, especially in patients with diabetes. This suggestion stemmed largely from studies on fat-deficient animals by other workers [*18*], who demonstrated an atypical form of acute medullary necrosis in some of these animals. Robbins and Angrist depicted the initial event as a rapid infarct-like necrosis of the pyramids precipitated by the infectious process, followed by reactive inflammation.

A concise review of renal medullary necrosis by Mandel [*19*] appeared in 1952. From a survey of the world literature he presented 160 cases in tabulated form. Of these patients ninety-six had diabetes. Of the remaining sixty-four patients, forty-eight had evidence of obstruction of the urinary tract. Mandel contended that the etiologic factor was simultaneous occlusion of multiple small renal blood vessels, precipitated by a combination of toxic and infectious factors. In the same year Knutsen [*20*] reported sixteen cases, emphasizing that infection and vascular sclerosis are the factors most often observed in fatal cases.

Simon in 1955 [*21*] made a survey of the postmortem material at the Mayo Clinic and gathered together forty-two cases and in addition tabulated 211 cases from the medical literature. The majority of patients in his own series, unlike the usual experience, did not have diabetes mellitus. He noted acute pyelonephritis in 76 per cent of the cases and acute and/or chronic pyelonephritis in 95 per cent of the cases. This point deserves emphasis, for it demonstrates that classification of patients into those with or without diabetes, and those with or without obstruction of the urinary tract obscures a more vital fact, namely, that pyelonephritis, irrespective of etiology, is the most common predisposing cause of renal medullary necrosis. This predis-

TABLE VI
INCIDENCE OF PYELONEPHRITIS AND RENAL MEDULLARY NECROSIS
(POSTMORTEM SERIES)

Author*	Period	Autopsies	Selection at Autopsy	Pyelonephritis (%)	Medullary Necrosis (%)
Ophuls [24]	1900–1923	3,000	None	4.8	...
Edmondson [16]	1924–1945	31,141	Non-diabetics	3.2†	0.07†
Aye [25]	1940–1952	1,715	Non-diabetics	0.7†§	0.17†
Wiesel [26]	? –1950	1,000	None	...	0.60
Schourup [22, 23]	1951–1955	5,005	None	...	1.05
Smith [27]	1908–1949	219‡	Diabetics only	5.9	2.7
Edmondson [16]	1924–1945	859	Diabetics only	12.5	3.4
Whitehouse [28]	1950–1955	266	Diabetics only	...	4.1
Robbins [17]	1932–1942	307	Diabetics only	19.5	5.2
Aye [25]	1940–1952	97	Diabetics only	14.4†	7.2†
Cates [30]	1931–1956	117	Diabetics only	8.0	...
Baldwin [31]	1934–1939	143	Diabetics only	21.7	...
Simon [21]	1934–1954	42	Medullary necrosis only	95.2	...

* Senior author only.
† Acute pyelonephritis only.
‡ Histological slides not available for a few patients.
§ Includes only patients over the age of twenty years.

position is due not only to compression of pyramidal blood vessels by inflammatory exudates, but also to the nephrosclerosis associated with chronic pyelonephritis. In the majority of cases in which careful histological examination is made, both inflammatory and vascular predisposing factors are found.

Recently, Schourup [22] reported fifty-three cases and proposed a new classification, dividing all patients into an "infectious" form (Type I), a "sclerotic" form (Type II), and a combination of these two types (Type III). Her proposal substitutes a pathological classification for previous clinical classifications but she maintains that such a division of patients is often clinically apparent. Type I (infectious) is characterized by acute signs and symptoms of urinary tract infection. There is equal distribution between the sexes, and between diabetic patients and those with obstruction of the urinary tract. At necropsy these patients are found to have bilateral lesions of acute pyelonephritis and often of cystitis. Hypertension is an infrequent finding in this group and only a minority die in uremia. Histologically there is little evidence of chronic renal disease. Thirty-two of Schourup's patients were so classified. Twelve of the remaining patients were included in her type II (sclerotic) category. Hypertension and cardiomegaly were more common in this latter group. Debilitation and short term illnesses were the rule. The majority of the patients died in uremia. At postmortem examination small granular kidneys with few signs of acute inflammation were noted. Important to her classification was the fact that most of these patients displayed signs of chronic renal disease, as evidenced by numerous hyalinized glomeruli, marked fibrosis and nephrosclerosis. Her type III category (I and II) included a few patients characterized by fatal exacerbation of an underlying chronic renal disease. From her records she noted that only those in the type I group, as would be expected, stood to benefit from operative intervention. Her type I group largely includes those formerly classified clinically as acute and subacute cases, while her type II would include those previously classified as chronic and/or asymptomatic. In a later communication [23], attention was drawn to the occurrence of medullary necrosis as a sequel to acute pyelonephritis, especially in young females, without diabetes mellitus or obstruction of the urinary tract.

Incidence. Table VI summarizes the autopsy series [21–31] listing the percentage of the total number of patients found to have pyelonephritis

and the percentage showing medullary necrosis, both unilateral and bilateral. Inspection of these figures reveals that pyelonephritis has been found in 0.7 to 4.8 per cent of the general population, whereas patients with diabetes have an unquestionably higher incidence, with percentages varying from 5.9 per cent to as high as 21.7 per cent. Sharkey and Root in 1935 [*32*] were among the first to call attention to the increased susceptibility of diabetic patients to infections of the urinary tract. They found evidence of urinary tract infection in 18 per cent of approximately 200 diabetic subjects at autopsy. The average duration of diabetes in these patients was 9.7 years. That hyperglycemia and glycosuria *per se* may predispose to infection was emphasized by Baldwin and Root in 1940 [*31*], from their analysis of eighty-six diabetic patients with renal infections of whom thirty-seven died. Robbins and Tucker [*29*] found that acute pyelonephritis was 4.5 times more frequent a cause of death in diabetic than in non-diabetic patients. Of their diabetic group 55 per cent had associated nephrosclerosis and 28 per cent had intercapillary glomerulosclerosis. In 1942 Harrison and Bailey [*14*], in a study of urine cultures from diabetic and non-diabetic patients, concluded that the offending organism was less important in the genesis of medullary necrosis than were the local tissue factors at the site of the necrosis, a view supported by Robbins, Mallory and Kinney [*15*]. The latter authors stated that the severity of the diabetes likewise was not an important etiologic factor. Their data indicated that at autopsy approximately one of four diabetic subjects with acute pyelonephritis has associated medullary necrosis.

Renal medullary necrosis has been found in less than 1 per cent of the general population as compared with 2.7 to 7.2 per cent of the diabetic population. Thus the increased incidence of pyelonephritis in diabetic patients is accompanied by a parallel increase in the incidence of medullary necrosis.

Edmondson, Martin and Evans [*16*] found that diabetic patients with acute pyelonephritis were predisposed to the sequelae of medullary necrosis if they were females, or if they were admitted in coma, or if they had Kimmelstiel-Wilson disease and/or nephrosclerosis. In nine of the eleven diabetic patients reported on by Whitehouse and Root [*28*] in whom medullary necrosis developed, there was a history of indwelling catheterization.

In an extensive review of medullary necrosis in 1958, Jornod [*33,34*] concluded that this disease is actually less frequent in diabetic subjects if a distinction is made between the medullary necrosis secondary to a necrotizing pyelitis with inflammatory destruction of the bordering pyramids and the medullary necrosis arising from within the renal pyramids; a distinction which, as he pointed out, is not yet generally accepted. He further suggested that the high incidence of diabetes mellitus in reported American cases may represent selection at autopsy.

Recently the question has been raised as to whether or not medullary necrosis is actually increasing in incidence. Pedersen and Schwartz diagnosed one, seven and twelve patients in three successive years [*35*]. That persons with diabetes are living longer than formerly is indisputable, as is the fact that since renal medullary necrosis is a disease of the older age groups, more diabetic subjects will now be afflicted. In addition, more diabetic patients are undergoing surgery now, which again may lead to an increased incidence of urinary tract infections and medullary necrosis. Another factor causing this apparent increase is the greater use of roentgenography for the diagnosis of this disease entity. However, we believe that the increased incidence in the past few years is more apparent than real because of the greater interest in the disorder. Pathologists interested in this entity, on reviewing previous histological specimens, often detect cases of medullary necrosis previously not recorded; for example Aye [*25*] reviewed specimens from diabetic patients and patients with pyelonephritis and discovered seven cases which were not reported over a thirteen year period.

In the European and Scandinavian medical literature attention presently is being focused on medullary necrosis as a nenhropathy attributable to the chronic abuse of phenacetin and related compounds [*36–40*]. The renal lesion has been described as a "primary chronic interstitial nephritis" resembling chronic pyelonephritis, but is said to be associated with a higher incidence of medullary necrosis. However, many of the patients reported on appear to have typical chronic pyelonephritis who coincidentally are habituated to phenacetin compounds.

Experimental. In the laboratory, medullary necrosis has been generally produced in three ways: (1) by creating an artificial obstruction in

the urinary tract and superimposing on this an induced bacteremia with consequent pyelonephritis; (2) by the injection of toxins; and (3) by metabolic means.

Pyelonephritis was first produced in animals, by means of ureteral obstruction, by Albarran and Halle in 1888 [*41*], who injected organisms into a ligated ureter. This work was confirmed by many subsequent authors [*42–44*]. Lepper [*45*] demonstrated that even transitory obstruction could be important in the genesis of pyelonephritis. Mallory, Crane and Edwards [*46*] produced unilateral pyelonephritis in 75 per cent of rabbits with unilateral ureteral ligation after the intravenous injection of colon bacilli. The first histological changes noted were "masses of bacilli in the capillaries and venules of the pyramids." This was immediately followed by endothelial tubular necrosis, an inflammatory response, and then development of interstitial abscesses. In some of their animals renal medullary necrosis developed very similar to that found in human subjects. It should be pointed out that the human kidney is multipyramidal whereas the rabbit kidney is monopyramidal; the rabbit kidney therefore is more vulnerable to a fulminating medullary necrosis, since necrosis of its single pyramid results in loss of the whole kidney.

Muirhead, Vanatta and Grollman [*47*] ligated the ureters of dogs, in some experiments bilaterally, in others unilaterally in conjunction with contralateral nephrectomy. The dogs were then dialyzed on an "artificial kidney" to prevent death in uremia before the histological changes of medullary necrosis could occur. Sixty per cent of the dogs showed papillary hemorrhages, in 36 per cent pyelonephritis developed, and in 32 per cent either coagulative or suppurative medullary necrosis developed. On histological examination dilatation of all tubules and bloodless, compressed glomeruli were noted (an infrequent finding in human medullary necrosis). These authors injected India ink in their experimental animals and in 50 per cent of the animals noted absence of the ink in the inner portion of the medulla, especially the tips of the papillae, while the cortex was well stained. This suggested that the increased intrapelvic pressure had altered renal hemodynamics, with curtailment of blood flow to the papillary tips. They considered their results in animals to be analogous to renal medullary necrosis in non-diabetic persons with obstruction of the urinary tract. They stated that the medullary zone of the kidney has a greater proportion of loose interstitial tissue than does the cortex, thus making the medullary area more susceptible to increased intrapelvic pressure as transmitted through the collecting system and renal tubules.

As was mentioned earlier, even transitory ureteral obstruction has been sufficient in some cases to induce ipsilateral pyelonephritis, and minor degrees of urinary tract obstruction may be associated with major infections. It is surprising that, with the large number of patients with known hydronephrosis, only a fraction have ever been shown to have medullary necrosis.

Recently, radioactive material instilled into hydronephrotic kidneys has been shown to be detectable in the blood [*48*], demonstrating that reabsorption of pelvic contents can occur, possibly by pyelotubular backflow. Murphy and co-workers [*49*] injected suspensions of Staphylococcus aureus and E. coli into the renal pelvis of dogs, then ligated the ureters and obtained samples from lymph (cisterna chyli), renal and femoral vein blood. They noted that if a bacteremia occurred, bacteria always appeared in the lymph, and at times the bacteria appeared in the lymph before the blood cultures became positive. Later studies [*50*] suggested that the venous outflow from the pelvis may be of greater importance than the lymphatic drainage.

Edmondson, Martin and Evans [*16*] were unable to reproduce medullary necrosis in depancreatized rats with prolonged glycosuria in the presence of ligated ureters, raising the question whether the increased susceptibility of diabetic subjects to infection is really due to hyperglycemia and glycosuria. Some have attributed this predisposition to dehydration in patients with poorly controlled diabetic states [*51–53*]. To pursue the point further, Marble, White and Fernald [*54*] suggested that the increased incidence of urinary tract infections in diabetic subjects was not the result of an increased susceptibility to infection, but due rather to predisposing urinary tract lesions (including bladder paresis) which they found in 78 per cent of their patients.

Thal [*55*] injected staphylococcal toxin intravenously into rabbits and noted that in all animals focal necrotic areas appeared in the medulla. Injection of toxin-antitoxin mixtures failed to produce this lesion. However, the renal changes differed from those occurring in man,

since practically complete renal cortical necrosis also occurred. Gross bilateral cortical necrosis is extremely rare in patients with medullary necrosis, but has been reported [*110*].

Gorrill [*56*] injected staphylococcal bacteria intravenously into mice and found that many of the mice lost one kidney completely ("bacteriologic nephrectomy") due to loss of their single renal papilla. The renal damage was proportional to the logarithm of the injected dose. De Navasquez in 1950 [*57*] found that only coagulase-positive staphylococci are capable of producing pyelonephritis in the unobstructed kidney. Intravenous injection of gram-negative bacteria did not produce pyelonephritis in the unobstructed kidney. In 1956 De Navasquez [*58*] injected E. coli and pseudomonas organisms into the ear veins of rabbits in whom renal scars had previously been produced by means of intravenous injections of S. aureus. Within forty-eight hours the gram-negative organisms were recovered from the urine, which contained pus and albumin, and was diminished in volume. The animals died of uremia between the sixth and thirteenth days. Postmortem study of the kidneys showed asymmetrical contraction due to coarse and irregular scars, accompanied by linear streaks of suppuration converging and extending to the renal pelvis. Linear abscesses were concentrated in the vicinity of the scars previously produced. The pelvis was inflamed only when confluence of the abscesses caused suppurative necrosis of the papillae. (Renal scars in man are considered to be areas of replacement fibrosis and are either congenital, ischemic or inflammatory in origin. They have been noted to increase in number with increasing age and in hypertensive patients) [*59*]. It would thus appear that gram-negative bacilli do not produce infection in the unobstructed kidney but they will do so in kidneys with intrarenal cortical scars. A clinical counterpart might be those patients with chronic renal disease (and hence intrarenal scarring) who through surgical intervention or manipulation of the urinary tract are subjected to a transient gram-negative bacteremia which could result in medullary necrosis by virtue of coalescence of abscesses at the bases of the pyramids, with consequent ischemia and necrosis. The most common portal of entry for diabetic patients with gram-negative bacteremia is the genitourinary tract and the chief offending organism has been the E. coli bacillus [*60,61*].

Rocha, Guze, Freedman and Beeson [*62,63*] investigated the possibility that different anatomical regions of the kidney may have variable vulnerabilities to bacillary infection. Simple intravenous injection of coliform bacteria without prior damage to the renal tissue failed to produce renal infection in rabbits, whereas with prior injury to the medulla by cauterization, followed by bacterial injection, pyelonephritis was found in 85 per cent of the animals. Medullary injury in association with instillation of bacteria into the bladder likewise gave a high incidence of pyelonephritis. Upon direct inoculation of bacilli of the colon into the cortex, more than 10,000 bacteria were needed to produce infection in the form of cortical abscesses, while less than ten bacilli were necessary to cause medullary infection upon direct medullary puncture; the latter lesions were characteristically wedge-shaped. The increased susceptibility of medullary tissue was attributed to localized obstruction of the collecting ducts, probably due to the technic of medullary puncture.

Medullary necrosis has also been reproduced in animals by the injection of various toxins. Vinylamine [*64*], tetrahydroquinone [*65*] and bromethylaminbromhydrate [*66*] have been used for this purpose. Mandel and Popper [*67*] in this country injected vinylamine intravenously into rabbits. The initial event produced was a medullary hyperemia with a cortical ischemia, followed by ischemic medullary necrosis. Sections revealed the formation of multiple thrombi. The primary effect of the toxin was considered to be a neurovascular one, with vasoparalysis, hyperemia and ischemia. As noted by Mandel and Popper, a direct nephrotoxic action of the vinylamine could not be excluded.

Another method which has produced an experimental variant of medullary necrosis is dietary restriction of fat. Borland and Jackson [*18,68*] fed rats a fat-free diet and on later microscopic examination of the kidneys of sacrificed animals noted fatty casts in the medulla and, in extreme cases, medullary necrosis. Cortical peritubular calcification was a prominent finding in all cases, and calcification of the medulla was found in the animals with medullary necrosis. It has been suggested by Robbins and Angrist [*17*] that the abnormal fat metabolism of diabetic persons may play a part in the genesis of their variant of medullary necrosis. It should be noted, however, that

the diets given were completely fat-free and also that the characteristic calcification noted on microscopic examination is an infrequent finding in human subjects.

The difficulty in assessing the significance of experimentally produced renal lesions is demonstrated by the report of White and Mori-Chavez [*69*] who administered mono-N-methylaniline to rats for a period up to one and a half years in the hope of producing hepatic tumors, but on sacrificing the animals found hyaline thrombosis of the smaller renal vessels with minimal inflammatory changes and bilateral medullary necrosis.

CLINICAL FEATURES

A detailed analysis of the cases reported in the world literature was made. The 250 cases selected for inclusion in this review [*70–151*] all were histologically verified.

Age Distribution. Medullary necrosis is more common in the older age groups, 54 per cent of the patients being over sixty years of age and 89 per cent over forty years of age. Only four patients under the age of twenty were noted. The disease has been reported in infants [*73,139,150*].

Sex. Sixty-five per cent of the patients were female, and 35 per cent were males.

Diabetes. Verified diabetes mellitus was present in 57 per cent, 43 per cent of the patients did not have diabetes. Of the diabetic patients 86 per cent had their disease less than fifteen years; 32 per cent less than five years. Only one patient had diabetes for more than twenty-five years. Of the forty-five cases recorded with sufficient data to estimate the adequacy of diabetic control, forty-three were classified as "poorly" controlled.

Presenting Symptoms and Signs. The majority of patients (70 per cent) presented with signs or symptoms referable to the urinary tract or with more generalized signs of sepsis (chills, fever, and the like). The three most common presenting symptoms, in order of frequency, were pain in the flank, chills and fever, and abdominal pain. Typical renal colicky-type pain was infrequent but when present generally signified passage of a sequestered necrotic papillae, occasionally with transient ureteral obstruction.

Hypertension. Because of the older age groups involved, a mean blood pressure in excess of 130 mm. Hg on admission was considered to be hypertensive. Only 25 per cent of the patients with recorded readings were hypertensive by this criterion. However, many patients were critically ill on admission, with blood pressure readings presumably lower than their usual values.

Race. The vast majority of patients were Caucasians but this appeared to reflect the clinical material available and not racial selection.

Cause of Death. The chief cause of death was uremia, which accounted for 64 per cent of the mortalities. Of those patients dying in uremia, in 90 per cent this was directly precipitated by medullary necrosis. The remaining 10 per cent had antecedent or associated renal disease (other than medullary necrosis) which lead to death. Septicemia accounted for 13 per cent of the deaths.

Acute Renal Failure. There were forty patients with unequivocal acute renal insufficiency. Undoubtedly, the true number was much higher since the data recorded in many cases was insufficient to determine if acute urinary suppression had occurred.

Catheterization. In twenty-eight cases there was a history of urethral catheterization immediately preceding the onset of clinical deterioration. Undoubtedly, a much larger number of subjects were catheterized but this was not recorded. Abuse of the catheter undoubtedly was a precipitating cause in many cases. Some patients with diabetes had been catheterized simply to obtain fractional urine specimens, and all too often diabetic patients admitted in coma had been catheterized either to estimate the residual urine or to obtain adequate urine for routine analysis. This practice cannot be too strongly condemned.

Clinical Classification. Patients were classified as "acute" if the onset was sudden, with toxicity, oliguria and fulminating uremia. Acute renal failure was common, and included the patients with shortest survival times and highest mortality rates; 50 per cent of the patients were so classified. This category includes the classic cases of medullary necrosis, i.e., the diabetic patient in whom severe urinary tract infection develops which rapidly leads to acute renal failure and death in uremia.

Thirty-four per cent of the cases were classified as "subacute." These cases were characterized by recurrent severe infections of the urinary tract, sometimes punctuated by the voiding of papillae, but generally with survival for months. Many of these patients had acute pyelonephritis

superimposed on chronic renal disease. The group included a number of patients with obstructions of the urinary tract.

The "chronic" or "asymptomatic" group included patients known to have medullary necrosis, generally by roentgenography and/or examination of voided tissue, who survived for years. The asymptomatic patients were usually dying of unrelated causes, and were found at postmortem study to have an unexpected medullary necrosis, often unilateral, and often in the healed stages. Sixteen per cent of the cases were represented in this category.

Roentgenography. Both intravenous pyelograms and retrograde pyelograms were determined in twenty-seven patients. In fourteen of these, the roentgenograms were considered sufficiently characteristic for diagnosis before death.

Diagnosis Before Death. Only 13 per cent of the patients were diagnosed before death. Roentgenography was the chief means of correct diagnosis, followed closely by histological examination of voided tissue and diagnosis based chiefly on clinical features.

The chief reason for failure to diagnose was failure to include the disease in the differential diagnosis although, in retrospect, a correct diagnosis might have been made on the basis of clinical features alone in the majority of cases. Many errors arose in assuming that diabetic patients in coma who failed to respond clinically to insulin therapy and hydration probably had had a cerebrovascular or myocardial infarction. Renal insufficiency should be considered more often in all patients in diabetic coma, especially those who respond poorly to therapy and those in whom the hyperglycemia and acid-base balance are not sufficiently deranged to account for the clinical state. The diagnosis would be made more often also if the urine specimens of all patients with a clinical picture consistent with medullary necrosis would be strained daily for voided papillae.

Obstructive Uropathy. Thirty-one per cent of the patients had obstruction of the urinary tract, in the majority of cases due to an enlarged prostate or to unilateral ureteral obstruction. In fifteen patients there was unilateral ureteral obstruction in conjunction with unilateral medullary necrosis on the same side. Both diabetes and obstructive uropathy were present in eighteen patients. Twenty-four per cent of the patients had neither diabetes nor obstructive uropathy. This latter group generally had severe renal vascular disease with superimposed infection or overwhelming septicemia.

Laboratory Data. Most patients on admission had a moderate leukocytosis, generally less than 20,000 white blood cells per cu. mm., with an associated anemia. Urinalyses showed a trace to 1-plus protein, fixation of specific gravity, pyuria and hematuria. Proteinuria in excess of 4 gm. per day was distinctly rare (four cases). Hematuria was usually noted early in the course of the disease and often disappeared; this may bear some relationship to necrosis of the pyramids with disruption of the associated blood vessels. Pyuria often was massive. Bacteriuria almost always was present when looked for. Of the microorganisms cultured from the urines in 143 cases, E. coli, staphylococci, proteus and streptococci accounted for 38 per cent, 18 per cent, 13 per cent and 9 per cent, respectively. "Mixed" infections were most common with obstruction of the urinary tract. There was no significant bacteriological difference between the positive urine cultures of diabetic and nondiabetic subjects; E. coli and staphylococci were the principal offending bacteria in both groups.

The erythrocyte sedimentation rate almost always was elevated and often greatly elevated (75 to 125 mm. per hour).

Half of the patients on admission had an elevated blood urea nitrogen (greater than 20 mg. per cent) or non-protein nitrogen (greater than 40 mg. per cent). Nitrogen retention is not an unusual finding in diabetic coma [*152*]; it is often, at least in part, a reflection of dehydration with resultant diminished renal blood flow and glomerular filtration rate [*153*]. If the azotemia persists, infection, obstructive uropathy and preexisting chronic renal disease should be considered as causative factors. Trever and Cluff [*154*], in a review of the records of 476 patients admitted to the Johns Hopkins Hospital in diabetic acidosis, recorded twenty-one patients with persistent azotemia attributable to infection or chronic renal disease.

Coburn in 1930 [*155*], and McCance and Lawrence in 1935 [*152*], described ketonemia in the absence of ketonuria in patients with diabetic acidosis as an indication of impending renal failure. This would appear to us to be useful in the diagnostic consideration of renal medullary necrosis in particular.

The azotemia of patients with obstructive uropathy appeared to be more closely related

to superimposed renal infection than to the obstruction *per se*. Many patients had marked azotemia with minimal prostate enlargement. Nitrogen retention directly due to obstruction of the urinary tract can often be differentiated from medullary necrosis by more complete anuria (less than 100 cc. per day), a palpable distended bladder, and by absence of signs of infection [*156*].

Survival. There were eighteen patients recorded as surviving histologically verified medullary necrosis.

Kidneys. Medullary necrosis was bilateral in 75 per cent of the cases, unilateral in 25 per cent. There was no preference for either side. Bilateral involvement was as common in the diabetic as in the non-diabetic groups. Of the fifty-four subjects in whom combined weight of the kidneys were recorded at autopsy, thirty-two were in excess of 400 gm. and fifteen were in excess of 600 gm. Diabetic glomerulosclerosis was found in 20 per cent of the kidneys of diabetic patients.

Renal clearance studies in medullary necrosis were reported by Edvall [*157*] who found a normal or low inulin to PAH clearance ratio in eight patients, in only three of whom the diagnosis was established by tissue examination.

Diagnosis of Medullary Necrosis. The chief clinical characteristics of the disorder are a urinary tract infection superimposed on diminishing renal function, especially in patients over forty years of age. The infection of the urinary tract most often is a fulminating pyelonephritis. The diagnosis should be strongly considered in any patient with a sudden toxic course following genitourinary tract instrumentation or surgical intervention, especially if there is clinical evidence of pre-existing renal disease (chronic pyelonephritis or glomerulonephritis, diabetic glomerulosclerosis, nephrosclerosis). The disease should be suspected in diabetic patients with disproportionate acidosis or who are in coma and respond poorly to treatment, or who respond to therapy but have persistent progressive azotemia.

The patients will generally present with signs referable to the urinary tract, such as dysuria and lumbar pain. Chills and fever are common. A moderate leukocytosis with anemia and an elevated erythrocyte sedimentation rate are the rule. Bacteriuria, pyuria, hematuria, albuminuria, and cylindruria are invariably present at some time in the course of the disease. The patient with diabetes will usually give a history of "poor control," with past or present episodes of coma, gangrene or skin infections.

Percutaneous renal needle biopsy may be of value in diagnosis, particularly in documenting patients who survive as did our patient (Case VI). Biopsy specimens which are negative are of little aid since medullary necrosis may be unilateral and may involve only a few papillae. A case in this series had been previously reported in the literature as an instance of Kimmelstiel-Wilson's disease diagnosed by renal biopsy [*83*].

Roentgenography. The value of pyelography in establishing the diagnosis of renal medullary necrosis was first stressed by Gunther [*12,13*]. Until recently only a few sporadic reports on the feasibility of diagnostic roentgenology appeared, attention previously having been focused on pneumopyonephrosis and cystitis emphysematosa (and associated pneumaturia) as roentgen signs of acute infection of the urinary tract in diabetic subjects.

Gunther [*12,13*] considered the earliest identifiable lesion to be a deformity of the calyceal fornix. This implies a localized loss of mucosal continuity at the fornix, so that a small amount of contrast media enters the renal substance (grade I). As the necrosis progresses the calyces become more distorted, "moth-eaten," and contrast medium penetrates deeper into the necrotic tissue. The path of this radiopaque penetration is quite uniform. The dye travels parallel to the outer surface of the kidney and penetrates from the fornices of a pyramid toward its mid-point, giving the appearance of ragged penetrating sinuses extending from the calyces into the pyramids. As the necrotic zone of demarcation becomes complete, the arc-like paths of contrast media from opposite fornices unite to form the familiar "sling" or "ring" shadow. When the pyramid is completely necrotic at its base it may detach completely (sequestered papilla) and if it does so the opaque medium fills the cavity left by the departed papilla, giving a "club-shaped" appearance. If the sloughed papilla remains in the pelvis it may become encrusted with calcium salts, or it may undergo dissolution. There are reports of pyelotomy for idiopathic calculi which on microscopic examination prove to be sequestered papillae.

Wall [*78*] noted that the renal outline is generally enlarged in cases of medullary necrosis, which he attributed to the inflammation of the

parenchyma. His report was followed by that of Ottoman et al. [*79*] who described the various roentgen findings indicative of this disease. As "suggestive" roentgenologic signs they listed calyceal haziness, ulcerative or erosive papillary changes, sinus formation, "ring" shadow, amputated calyces, club-like papillary fossa, moth-eaten infundibula and intrapelvic filling defects. At the same time several articles appeared in the Scandinavian literature [*103,111,113*] pointing out the feasibility of roentgenological diagnosis. Christoffersen and Anderson [*111*] noted that the roentgenographic diagnosis is often retrospective and that many of the aforementioned changes are minor and equally suggestive of other diagnoses, especially tuberculosis. Also, various "backflow" phenomena [*164,165*] such as fornix sinus and pyelolymphatic, pyelovenous and pyelotubular backflow may mimic the roentgenographic appearance of medullary necrosis, as may calyceal diverticula [*166*]. Lusted, Steinbach and Klatte recently described renal papillary calcification, best demonstrated by laminography, as a helpful roengtenographic sign [*83*]. Lagergren and Lindvall [*167*] report that of fifty-four patients with this lesion, they found "suggestive" changes in fifty-two. Thirty-eight per cent of their patients had calculus disease. Supporting the work of Lusted, Steinbach and Klatte [*83*], they point out that the calculi in these patients have identifying characteristics, namely, a nucleus of low density (sequestered papillary fragments) surrounded by a dense shell of calcium. It should be stressed that these roentgenographic signs are suggestive but not diagnostic of medullary necrosis.* The ring shadow probably is the most specific roentgenographic sign.

Before employing pyelography (especially retrograde) as a diagnostic aid in these patients one must consider the hazards which such procedures induce [*168–170*]. In three patients medullary necrosis developed after such retrograde examination [*25,74,91*]. Because this potential danger is life-threatening we believe that retrograde pyelograms are rarely indicated in patients thought to have medullary necrosis, and that intravenous pyelograms should be undertaken with great caution since most of these patients have rapidly deteriorating renal function.

* Hence patients with a diagnosis made solely on roentgenographic (and clinical) evidence were not included in this series.

Roentgenograms have been used to determine unilaterality in this disease, since surgical intervention is considered warranted by some investigators if medullary necrosis affects a single kidney. However, many cases in this review demonstrate clearly that all too often, despite roentgenological evidence of unilateral involvement, the patient succumbs shortly after operation and is found to have medullary necrosis of the remaining kidney. Schourup [*23*] in a post-operative follow up of fourteen patients, found that three had died six months later, and the remaining patients all had symptoms on the contralateral side, often with the passage of papillae.

Differential Diagnosis. Excluding overt obstructive uropathy, which generally becomes apparent in the initial clinical evaluation, and pre-existing renal disease such as chronic glomerulonephritis or pyelonephritis which may be evident in the past history, the principal problem in differential diagnosis is to distinguish acute tubular necrosis, renal medullary necrosis and renal cortical necrosis.

It is our belief that these three variants of acute renal insufficiency are sufficiently distinct from each other (both pathologically and clinically) to make correct diagnosis possible in the majority of cases on clinical features alone.

Acute tubular necrosis is due to renal ischemia most often caused by trauma, hypovolemic shock, mismatched blood transfusion, septic abortion or nephrotoxins. Its cardinal feature is hypotension. The clinical course of anuria, oliguria and diuresis is characteristic. Statistically, acute tubular necrosis is the most common cause of acute renal failure and, unless the patient is comatose, the precipitating cause is generally evident.

Renal cortical necrosis is most often associated with the mishaps of pregnancy, especially abruptio placentae. It is, therefore, characterized by marked female preponderance. It must always be considered in obstetrical cases of renal insufficiency. The triad of toxemia, abruptio placentae and bleeding disorders, when associated with acute renal failure, is almost diagnostic. Figure 6 shows the age, sex and etiologic distribution of 196 patients with cortical necrosis previously reported in the literature [*171,172*]. Next to this, for comparison, is a similar graph of 196 cases of medullary necrosis from this review. It is apparent that cortical necrosis is most common in the twenty to forty year age group as op-

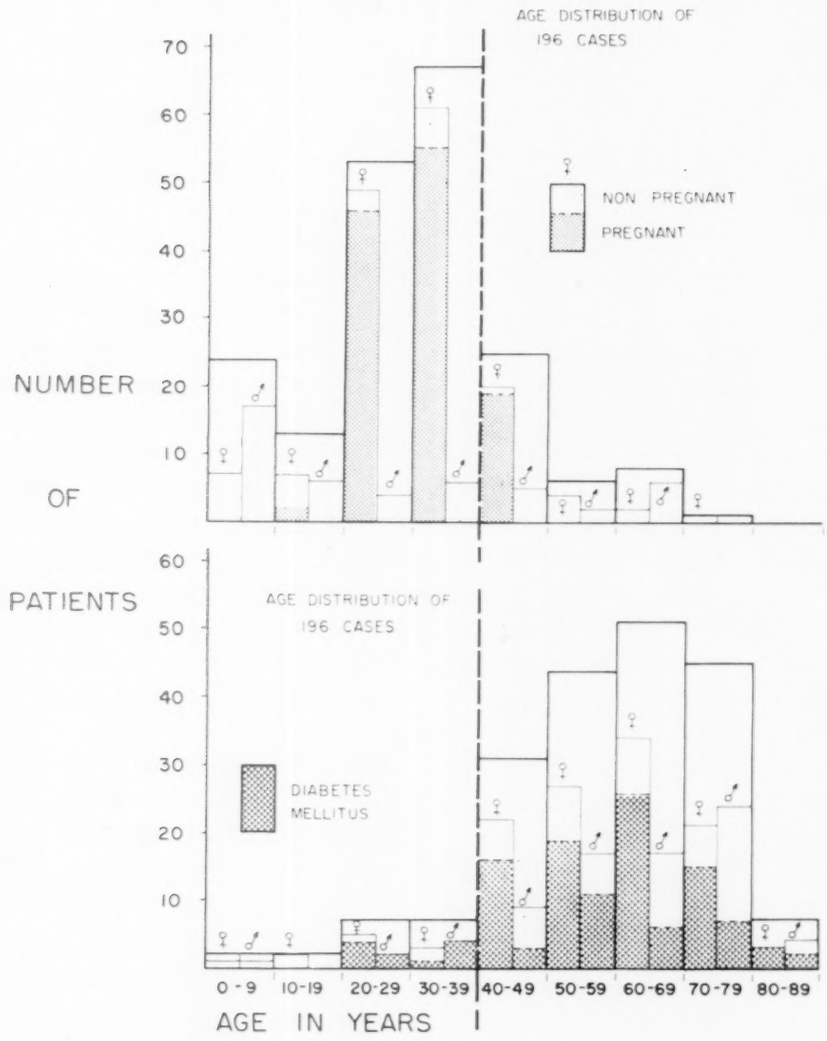


FIG. 6. Age distribution and number of patients with renal cortical necrosis (*above*) and renal medullary necrosis (*below*).

posed to the fifty to eighty year age group of medullary necrosis. It is just as evident that abnormal pregnancy is the leading accompaniment of cortical necrosis, whereas it is exceedingly rare as an accompaniment of medullary necrosis. Furthermore, cortical necrosis is more often present in infants and children, and is rare in woman after fifty years of age.

Figure 6 clearly shows that medullary necrosis is a disease of the older age groups, and that diabetes mellitus is often a prominently associated disease, regardless of age. It occurs more often in female diabetic patients. It is relatively uncommon in the patients reviewed herein under forty years of age. Its principal features are infection of the urinary tract and/or bacteremia in association with diabetes mellitus and/or obstructive uropathy.

Prognosis and Treatment. In a disease entity usually recognized in the past by the postmortem findings, it is difficult even to approximate the survival rate. On the whole, the prognosis is determined as follows: (1) by the delay in diagnosis in relation to onset of the disease; (2) by the therapy given (this includes medical correction of infection and/or surgical correction of known predisposing factors; and (3) by the rapidity of progress of any predisposing or associated disease state. The eradication of infection is the keystone of treatment. In the opinion of the authors all infections of the urinary tract should be considered and treated as active pyelo-

nephritis. Any infection of the urinary tract in a diabetic patient or in a patient with obstruction of the urinary tract must be considered potential medullary necrosis. Inherent in this implication is that one must avoid known inciting factors, especially urethral catheterization. A detailed program of antibiotic therapy should be undertaken, based on antibiotic sensitivity tests on the offending microorganisms obtained through "clean-catch" urine specimens. Any obstructive element must be surgically evaluated since at best, without removal of obstruction, a superimposed infection can only be suppressed and not eradicated. Nephrectomy is of dubious benefit since the disease is usually bilateral. That the disease is compatible with survival is strongly supported by many cases included in this review [*14,20,30,35,72,75,78,83, 86,98,104*], by the finding of healed lesions at examination at autopsy, and by our own experience with one patient (Case VI).

PATHOGENESIS

Various theories of pathogenesis of this disease have been proposed. These include confluent pyramidal abscesses [*15*], increased intrapelvic pressure [*1,47*], paramyloid deposits [*92*], increased interstitial pressure due to edema and/or inflammatory exudates [*1,47*], altered papillary musculature with contractions [*7,95*], constriction of minor calyces [*147*], pelvic reflux of urine [*16*] or contrast media [*10*], intercapillary glomerulosclerosis [*77*], vascular spasm [*7*], thrombus formation secondary to vasospasm [*6,13*], arterial and arteriolar sclerosis [*16,80*], papillary sclerosis [*151*], thrombosis or embolization of renal vessels [*80*], and bacterial [*23*] or drug sensitivity [*36*].

From this review, and the cases presented, infection emerges clearly as the common denominator in renal medullary necrosis. We have previously described pyelonephritis as a continuous clinical and histologic spectrum of infectious disease of the kidneys [*173*]. Medullary necrosis is best conceived to be one extreme of medullary pyelonephritis. The associated inflammation of sensitivity reactions, e.g., to contrast medium in one patient (Case II) may play a contributory role in some cases. The finding of coliform bacilli in all of our positive cultures and in 38 per cent of cultures reported in the literature might suggest some bacterial specificity. However, since these organisms account for one-fourth of the positive cultures in our Renal Clinic, it would be difficult to prove an association. Tissue factors in the host would seem to be the most important determinant.

It is our belief that renal medullary necrosis is a form of pyelonephritis which is more devastating than uncomplicated pyelonephritis because its natural course is altered by associated disease states. Among the principal factors causing the evolution and progression of this variant of pyelonephritis are the following: (1) the diabetic state and its enhancement of infections; (2) pre-existing renal disease, especially chronic pyelonephritis; (3) urinary tract obstruction, because it is a source of infection and notably of antibiotic-resistant infections; and (4) nephrosclerosis, which by compromising pyramidal blood supply makes the medulla more vulnerable to ischemia and subsequent infarction, and also because nephrosclerosis with its associated fibrosis of itself serves as a nidus for infection.

We view the disease process as originating with bacterial implantation in the interstitial tissue of the outer medulla and renal cortex. The route of infection (hematogenous, ascending, lymphatic) can rarely be ascertained. From the interstitial tissues the bacteria spread to cause necrosis of the adjacent tubular epithelium, and spill into the tubular urine. Active bacterial extension ensues, both within the interstitial tissue and intraluminally within the tubules. The inflammatory response on the part of the body to this infection is governed by poorly understood immunological and other host resistance factors. If the inflammatory response is poor, self-limited or restrained by local tissue factors, the infection becomes rampant, the small blood vessels are involved, hemorrhage and early necrosis follow. The result is a diminished pyramidal blood flow and consequent ischemia. As the interstitial pressure increases with increasing edematous formation, there is slowing of the blood stream and areas of stasis allow further bacterial proliferation, eventuating in necrosis of the pyramids. If the ischemia is severe and of acute onset, the necrosis will be of the ischemic-coagulative variety; if the ischemia is less severe and more prolonged, an exudative-coagulative necrosis will result. Thus we view medullary necrosis as generated by an uncontrolled infection, abetted by local vascular tissue factors and by defective resistance on the part of the host.

SUMMARY

Patients with diabetes and/or obstruction of the urinary tract and infection are predisposed to inflammation progressing to ischemic necrosis of the renal medulla, a life-threatening disease which has been described under the terms necrotizing papillitis, papillary necrosis and necrotizing pyelonephritis but here designated renal medullary necrosis. The present study is based on analysis of six patients and a review of the clinical and experimental literature on renal medullary necrosis.

A concept of the disease has been developed which emphasizes infection as the common denominator. The pathologic disorder of medullary necrosis is viewed as an uncontrolled infection, compromised by local vascular tissue factors and by defective resistance on the part of the host. Demonstration of this lesion on biopsy in a previously anuric patient suggests a prenecrotic phase of the disease which may be reversible with prompt antibiotic therapy.

ADDENDUM

A recent article by Baker [*174*] supports the concept that the renal papillae have a dual blood supply. In addition to the vasa rectae from the efferent arterioles of the juxtamedullary glomeruli, dissection of neoprene-injected human kidneys revealed tortuous muscular arteries arising from the interlobular arteries, and running to the minor calyces. These vessels are thought to supply the calycine mucosa and renal papillae. It is suggested by the author that these vessels protect the renal medulla from ischemic necrosis in cases of acute tubular necrosis, and furthermore that impairment of these calycine vessels, by pressure effects, occlusion, etc., in association with renal ischemia, may lead to renal medullary necrosis.

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Clinicopathologic Conference

Progressive Hepatic Failure

STENOGRAPHIC reports, edited by Lillian Recant, M.D. and W. Stanley Hartroft, M.D. of weekly clinicopathologic conferences held in the Barnes and Wohl Hospitals, are published in each issue of the Journal. These conferences are participated in jointly by members of the Departments of Internal Medicine, Preventive Medicine, and Pathology of the Washington University School of Medicine and by Junior and Senior medical students.

THE patient was a seventy-three year old white retired packing house worker who was admitted to Barnes Hospital for the third time on April 15, 1959, with the chief complaint of vomiting blood.

The patient entered the hospital on December 22, 1949 for excision of hemorrhoids which had been present for four years. Two months before admission he had an episode of chills and fever, slight cough and malaise, which was associated with a weight loss of twelve pounds. The patient had had jaundice at the age of fifteen and typhoid fever at sixteen. He admitted to drinking alcohol only occasionally. His father died of liver trouble at age seventy-nine.

Physical examination was within normal limits except for the presence of bronchial breath sounds over the right upper posterior portion of the chest, a small right indirect inguinal hernia and prolapsed internal hemorrhoids.

Urinalysis and blood counts were within normal limits, as were blood sugar and non-protein nitrogen. The blood serology was positive at 2 Kahn units. The result of the cardiolipin test was positive. The total serum protein was 6.5 gm. per cent with 2.5 gm. per cent albumin and 4 gm. per cent globulin; the cephalin cholesterol flocculation was 2 plus and the thymol turbidity was 20.8 units. On examination of the chest bilateral apical scarring was described by the radiologist. Roentgenographic examination of the upper and lower gastrointestinal tract was within normal limits, as was an electrocardiogram.

On proctoscopic examination the colon was normal. Hemorrhoidectomy was performed. The patient was discharged on January 7, 1950.

The patient felt well after leaving the hospital. Two years before his second admission on January 8, 1952, pedal edema developed. This was relieved by a low salt diet. One and a half years before this admission he noted a mass in the left breast which subsequently became firm and tender at intervals. He had lost 7 pounds in weight since his last admission.

Physical examination was non-contributory except for the presence of a firm mass in the left breast, 2 cm. in diameter.

The hemoglobin was 12.6 gm. per cent, the red blood count 4 million/cu. mm. The result of the cardiolipin test was positive in undiluted serum. The urine, white blood count and differential were normal.

A left mastectomy was performed and on histologic examination of the excised specimen a diagnosis of gynecomastia was made. The patient was discharged on January 13, 1952.

The patient felt well until about two years before his third admission on April 15, 1959, when he began to lose weight. Eleven months before this admission his family had noted a slight yellowish tinge to his skin. Six months before admission he became anoretic and abdominal swelling developed. About this time he was given "heart pills" by his physician but he became weaker and edema of the lower extremities developed. One month before admission he passed tarry stools; he entered another hospital where a paracentesis was performed and 1 unit of blood transfused. Barium studies did not disclose the site of bleeding. Eight days before admission he vomited black material and on the day of admission he vomited dark blood several times. His family stated that he had consumed 6 to 8 bottles of beer daily since his teens and that he had weekly bouts in which he drank a case of beer in the course of an evening. His appetite was good and the diet apparently adequate until the year before admission.

On physical examination the temperature was 37°c.; pulse, 100/minute; respirations, 24/minute; blood pressure, 100/50 mm. Hg.

The patient was a thin cachetic white man who was disoriented as to time. His skin was icteric; no spiders were present. There was palmar erythema and marked clubbing of the digits. He had a barrel chest; breath sounds were distant. The heart was not enlarged and no murmurs were heard. There was abdominal distention with prominent thoracoabdominal veins; shifting dullness and a fluid wave were present. The liver was hard and nodular; its edge was 2 cm. below the right costal margin and 5 cm. below the xiphoid. The right testis was atrophic. There was a hydrocele on the left. The prostate was slightly enlarged. There was pitting edema of the legs and thighs, and a rhythmic flapping tremor of the outstretched hands.

The laboratory data showed the hematocrit to be 23 per cent; white blood cells, 10,000/cu. mm. with 75 per cent segmented forms, 1 per cent basophils, 22 per cent lymphocytes and 2 per cent monocytes. Platelets appeared normal on the smear. The urine had a specific gravity of 1.012, a pH of 5, and contained a trace of protein but no sugar. Bile and urobilinogen were present in the urine. The stool was negative to guaiac testing although subsequent stools were strongly positive. The reaction to the cardiolipin test was weak in undiluted serum.

The blood urea nitrogen was 57 mg. per cent; blood sugar, 113 mg. per cent; total serum bilirubin, 5.5 mg. per cent with 2.7 mg. per cent indirect fraction, 2.8 mg. per cent direct; cephalin cholesterol flocculation test, 2 plus; thymol turbidity test, 6.4 units; total serum protein, 5.2 gm. per cent with 3.1 gm. per cent albumin, 2.1 gm. per cent globulin; glutamic oxaloacetic transaminase, 295 units; acid phosphatase, 4.4 units; alkaline phosphatase, 12.4 units; prothrombin time, 42 per cent of normal; and carbon dioxide 28.9 mEq./L.; serum sodium, potassium, chloride, pyruvic transaminase, prostatic acid phosphatase, calcium and phosphorus were normal.

The electrocardiogram was interpreted as "abnormal form of ventricular complex." A roentgenogram of the chest was interpreted as showing pulmonary edema. The venous pressure was 100 mm. of saline; decholin circulation time was thirteen seconds.

Despite initial therapy, which consisted of blood transfusion, oral administration of neomycin glucose and antacids, parenteral administration of vitamins including K_1 oxide, cathartics and enemas, the patient remained obtunded. The hematocrit rose to 34 per cent after 3 units of blood were given; the gastric aspirate was grossly bloody. On esophagoscopic examination, esophageal varices were seen, but no bleeding point was detected. A Sengstaken tube was placed in the stomach, but the balloons were not inflated. Two more units of blood were given. A paracentesis yielded straw colored fluid with protein content of 1.2 gm. per cent and 35 mononuclear cells/cu. mm.; the fluid was sterile on culture. Penicillin and streptomycin were administered. Because of evidence of poor tissue turgor, intravenous fluids were administered. On the second and third hospital days the serum sodium dropped to 124, then 114 mEq./L.; 250 ml. of 2½ per cent saline solution was given but lethargy persisted. Despite small amounts of blood in the gastric aspirate, the hematocrit was stable at 38 per cent.

The blood urea nitrogen remained about 40 mg. per cent, and oliguria was noted. Fifty per cent glucose in water at the rate of 500 to 800 ml./day was given. The urinary sodium excretion ranged between 1 and 3 mEq./day. Liver function studies made on the fifth hospital day showed the albumin to be 2.3 gm. per cent and the globulin 2.8 gm. per cent; otherwise the results were unchanged from the earlier study. Blood and urine cultures were sterile. By the sixth hospital day there was no further evidence of bleeding, and oral feeding of a low protein liquid diet was initiated. On the eighth day the patient aspirated a large quantity of vomitus and became cyanotic. A tracheostomy was performed. On the ninth day he passed a tarry stool; his hematocrit fell to 32 per cent. A Sengstaken tube was passed and the gastric balloon inflated; 2 units of blood were given. There was continued evidence of bleeding over the next four days; the blood urea nitrogen rose progressively to 98 mg per cent.

On the thirteenth hospital day the serum albumin was 1.9 gm. per cent, with 3.1 gm. per cent globulin; the cephalin cholesterol flocculation was 4 plus. Although there no longer was evidence of bleeding, the patient was quite weak, and his urinary output had fallen to 300 ml./day. Roentgenographic examination of the chest, performed on the fourteenth hospital day, was interpreted as showing a resolving pneumonia and subcutaneous emphysema; the possibility of a pneumomediastinum was raised. Over the next four days the patient remained

obtunded and became profoundly oliguric and hypotensive. He died on May 3, 1959, the nineteenth hospital day.

CLINICAL DISCUSSION

DR. SOL SHERRY: This man had a history of jaundice at age fifteen. He was a heavy beer drinker for most of his life. At the age of fifty-eight, he noted the appearance of hemorrhoids and at the age of sixty-two, about two months before his first admission to Barnes Hospital a febrile illness developed. This was associated with chills, some cough and a loss in weight. He recovered from this illness and was admitted to our surgical service for hemorrhoidectomy. At the time of admission, it was noted that the results of a number of hepatic function tests were abnormal and that the serology was positive in a low titer.

Following the hemorrhoidectomy he did well until the age of sixty-four when he noted some pedal edema, which cleared on a low salt regimen. Shortly thereafter he noted the onset of left-sided gynecomastia. About a year later, at the age of sixty-five, he was admitted for the second time for a left mastectomy. Unfortunately, no hepatic function studies were performed on this occasion. Surgical pathological examination of the breast at the time of mastectomy confirmed the diagnosis of gynecomastia.

The patient again did well until the age of seventy-one when he noted the onset of weight loss. A year later, at the age of seventy-two, slight icterus appeared and I assume this persisted until his final admission. About six months after the appearance of icterus, he noted the onset of anorexia, abdominal swelling and edema of the lower extremities which did not respond to "heart pills." His symptoms progressed until the age of seventy-three when he had an episode of melena. About three weeks later he had a bout of hematemesis and when this recurred eight days later he was admitted for the third and final time. His course in the hospital, which extended over a nineteen-day period, was complicated by severe recurrent bleeding, coma, aspiration, oliguria, renal failure and finally shock and death. Mr. Kreager will present the roentgenographic findings.

MR. JACK KREAGER: The only films we have available were taken during the patient's last admission to Barnes Hospital. They were of the chest and were non-standard portables because of the debilitated state of the patient. On admission, the examination showed that a Sengstaken gastric tube was in place and that the mediastinum and heart shadows were within normal limits. There was a diffuse, cloudy, infiltrate in the lung parenchyma, radiating out from the hilar regions bilaterally, and somewhat symmetrically. The peripheral one-third of the lung fields were fairly clear, although small areas of lobular emphysema were visible. The findings were considered to be pulmonary edema. A roentgenogram taken fourteen days after admission, and two days after the episode of vomiting and aspiration, showed the tracheostomy tube in place, and areas of radiolucency over each shoulder and in the neck. These were interpreted as subcutaneous emphysema. There was also an area of radiolucency in the mediastinum, compatible with mediastinal emphysema, although on an anteroposterior view, it was impossible to make this diagnosis definitively, because the air could have dissected along the chest wall and in the back. The heart had not changed in size. There was clearing from the previous films on the left, while on the right a diffuse nodular infiltrate persisted mostly in the lower part of the lungs. This was compatible with pneumonia secondary to aspiration. In summary, the findings are those of pulmonary congestion, later subcutaneous and possibly mediastinal emphysema, and finally aspiration pneumonia.

DR. SHERRY: Was there anything peculiar about the right diaphragm?

MR. KREAGER: It was slightly elevated, but this was probably due to the enlarged liver.

DR. SHERRY: Mr. Armstrong, let us begin with this patient's illness at the time of his first admission to Barnes Hospital. We have information at that time that results of hepatic function tests were abnormal, and we know from his subsequent course that he probably had extensive hepatic disease. Do you believe he had cirrhosis of the liver at that time?

MR. CLAY ARMSTRONG: Yes, I think so.

DR. SHERRY: What points make you favor cirrhosis of the liver rather than any other type of hepatic disease?

MR. ARMSTRONG: The prolonged course which the patient showed with features characteristic of cirrhosis such as edema, evidence of gynecomastia, hemorrhoids, bleeding, ascites and a history of a heavy alcoholic intake make cirrhosis of the liver quite likely, even at the time of hemorrhoidectomy. The results of liver func-

tion tests were certainly compatible with this diagnosis.

DR. SHERRY: Since I assume that you all agree with this interpretation; let us consider the etiology of the cirrhosis.

MR. ARMSTRONG: I think that this is the cirrhosis which is associated with alcoholism, probably not the postnecrotic type. None of the indications are good enough to suggest other types of hepatic disease.

DR. SHERRY: Why do you say that? Do you not believe that an individual with postnecrotic cirrhosis can exhibit a course of the type described here?

MR. ARMSTRONG: It is not possible to differentiate these two entities very readily. The absence of a history of hepatitis is certainly not of major help here since a large number of patients have anicteric hepatitis. If this disease began in 1949, I think that it ran a rather long course without earlier complications for a postnecrotic lesion.

DR. SHERRY: Mr. Eberstein, which etiologic factors did you consider seriously and what was your final conclusion?

MR. ALAN EBERSTEIN: I do not think we can wholly discount the fact that the patient had an episode of chills, fever and malaise two months before his first admission to Barnes Hospital. I believe this could well have been an episode of subicteric virus hepatitis. His abnormalities of liver function might have been a reflection of the convalescent stage of this illness. I am not convinced that his hepatic disease is due to chronic alcoholism at this advanced age.

DR. SHERRY: Is an individual with hepatitis required to have a bout of icterus in order to have cirrhosis of the liver develop?

MR. EBERSTEIN: No, he is not.

DR. SHERRY: Is it more common to find anicteric hepatitis followed by cirrhosis rather than icteric hepatitis?

MR. EBERSTEIN: I do not think that the presence or absence of jaundice aids in making a prediction as to the eventual course of the disease.

DR. SHERRY: If viral hepatitis was responsible for the cirrhosis, can we tell whether the cirrhosis in our patient was of the postnecrotic type or of the Laennec's type?

MR. EBERSTEIN: I do not think we can differentiate on the basis of liver function tests whether the cirrhosis is on a postnecrotic or on a Laennec cirrhosis basis.

DR. SHERRY: Is there any way to clinically distinguish between postnecrotic cirrhosis and Laennec's cirrhosis?

MR. EBERSTEIN: A recent series reported by Dr. Sheila Sherlock in London, compared a non-alcoholic group of patients with cirrhosis with an alcoholic group in Boston. The differences in the results of liver function tests were essentially negligible. The only differences in the two groups could be summarized as follows: The alcoholic group had larger livers and a more prolonged course characterized by remissions and exacerbations while the patients in the London group showed a progressive, relentless downhill course.

DR. SHERRY: Then from a clinical standpoint our patient could have a postnecrotic cirrhosis, or a Laennec's cirrhosis. If it is postnecrotic cirrhosis then a viral etiology is most likely; if it is a Laennec's cirrhosis then the etiology may be obscure. Mr. Jackson, what about an alcoholic cirrhosis in our patient? Is not the cirrhosis which follows alcoholism usually related to malnutrition? To our knowledge the patient always had an adequate diet.

MR. ED JACKSON: One would like to have a history of malnutrition but this is not always found. In fact, recent evidence suggests that alcohol itself may provide the metabolic environment which favors hepatic injury.

DR. SHERRY: Mr. Steiger, what types of hepatic injury do we see in chronic alcoholism?

MR. BARRY STEIGER: Alcoholics will frequently have fatty metamorphosis of the liver. This may manifest itself by an acute hepatic failure with death in a few short weeks. Biopsy might show extensive fatty metamorphosis and liver cell necrosis. On the other hand, a more prolonged course may be seen. A liver biopsy might show only a moderate amount of fat, fibrosis and inflammation. Then again, there may be a very prolonged course, such as was seen in this patient, with the eventual development of frank cirrhosis. The natural history of the progression is not known. Perhaps fatty metamorphosis in the beginning is the cause of this end stage cirrhosis.

DR. SHERRY: Can alcoholism alone cause hepatic damage or must nutritional factors be present?

MR. STEIGER: I do not think there is any evidence that alcohol alone can cause hepatic damage. There have been reports that alcohol can increase the requirements of the liver for essential substances, but I do not think there is

any good evidence that alcohol is toxic to the liver cell. Schmid has shown that with alcohol as a substrate increased formation of reduced nucleotides may occur and accelerate fat synthesis in the liver.

DR. SHERRY: I believe that Klatskin has been rejuvenating the idea that perhaps alcoholism *per se*, independent of nutritional factors, can induce hepatic damage. There is some experimental work to support this, and not infrequently, we see patients who have cirrhosis and an alcoholic history yet do not admit to any malnutrition. The possibility has been suggested that alcoholism may reactivate a latent viral infection in the liver, which may then lead to the development of cirrhosis. Our patient also had a positive reaction to the serologic test. Do you believe serious consideration must be given to syphilis as a cause of the cirrhosis?

MR. STEIGER: Despite the fact that in hepatic disease the globulins are strikingly elevated, the incidence of a biologically false positive reaction to the serologic test is quite rare. Tertiary syphilis could produce a clinical picture exactly like that presented by our patient. Healing of gumma produces cirrhosis which is frequently suggested by enlargement of the left lobe of the liver and by a low grade febrile course. For these reasons, I believe syphilis should at least be considered.

DR. SHERRY: Mr. Black, would you have given our patient antisyphilitic therapy for his hepatic disease?

MR. PAUL BLACK: I think one would like to have more well defined criteria for the diagnosis of syphilis prior to the administration of antisyphilitic therapy. A lumbar puncture and a more highly positive reaction to the serologic test would be desirable. I think the probability that this patient had syphilitic liver disease is small. Some authors say that 20 per cent of people with cirrhosis have positive serologic test, whereas of all subjects with cirrhosis, less than 5 per cent have cirrhosis on the basis of syphilitic cirrhosis.

DR. SHERRY: Mr. Schoentag, would you agree that the positive serologic test in our patient was simply a manifestation of a latent syphilitic process and had nothing to do with the cirrhosis?

MR. JOHN SCHOENTAG: Yes. As previously stated, an increased incidence of syphilis in cirrhotic patients has been noted.

DR. SHERRY: Do the results of hepatic function tests during the patient's first admission give us any clues as to the extent or activity of the cirrhotic process?

MR. SCHOENTAG: The low serum albumin indicates that the liver is no longer producing albumin; but high globulin, as far as I know, is not significant in evaluating the functional state of the liver.

DR. SHERRY: I agree that the low albumin would indicate that the synthetic capacities of the liver were reduced, and that a good deal of the functional reserve of the liver was gone. Mr. Sly, what about the high globulin level? Does this indicate anything to you?

MR. MICHAEL SLY: Globulin elevations are usually related to mesenchymal reaction and perhaps are highest during active processes. The globulins are thought to be produced by plasma cells within the bone marrow. We might infer from this that this patient did have a certain amount of hepatic fibrosis at this time and perhaps some activity of his hepatic disease.

DR. SHERRY: Hyperglobulinemia is usually associated with inflammatory changes in the liver, and this is often indicative of an active scarring process. Mr. Cooperman, what about the hemorrhoids? Do you believe they were related to the cirrhosis?

MR. LEE COOPERMAN: Hemorrhoids are seen more frequently in persons with cirrhosis because of portal hypertension and collateral circulation. However, they are so common in people of this age that I do not think one can say whether they were due to cirrhosis or liver disease.

DR. SHERRY: Two years after our patient is suspected of having active cirrhosis, pedal edema and gynecomastia develop. How frequently does gynecomastia occur in cirrhosis?

MR. COOPERMAN: I believe the incidence is quite high, of the order of 10 to 20 per cent. The gynecomastia is said to be due to the inability of the liver to conjugate estrogen, so that circulating levels of estrogen remain quite high. There may also be other hormonal effects since testicular atrophy occurs and estrogenic activity is then unopposed.

DR. SHERRY: Has it been described as occurring unilaterally as in this individual?

MR. COOPERMAN: Yes, it can be unilateral or bilateral.

DR. SHERRY: The presence of spiders in cirrhosis is usually considered a sign of active disease, and the prognosis is usually poor in those who have lots of spiders. Does gynecomastia have the same significance?

MR. COOPERMAN: I do not know, but I would imagine, since spiders and gynecomastia may reflect the same hormonal changes that the prognosis might be the same in both cases.

DR. SHERRY: I would agree that gynecomastia has the same type of significance that we ordinarily attribute to spiders. Mr. Fogg, are you satisfied with the usual explanation that gynecomastia is due to a lack of estrogen inactivation?

MR. LYMAN FOGG: Yes, plus the fact that testicular atrophy is commonly seen with gynecomastia as was noted in this case. Thus a general decrease in androgenic hormones may also play a role.

DR. SHERRY: Yes, it has been pointed out that the gynecomastia may be a consequence of the testicular atrophy. It has been suggested that the sequence of events is increased estrogen levels due to failure of inactivation, pituitary suppression of gonadotropins, testicular atrophy and finally gynecomastia. Mr. Boren, what about pedal edema occurring in subjects who have cirrhosis without ascites? What is the mechanism of the fluid retention under these circumstances.

MR. CHARLES BOREN: There appear to be many factors which contribute to the formation of edema in cirrhosis. One of these relates to the effect of hypoalbuminemia with subsequent decreased colloid osmotic pressure of plasma. Additional factors may be hormonal. Aldosterone excretion is increased in cirrhosis and must be classified as secondary aldosteronism. This hormone increases the reabsorption of sodium ion. Another factor is antidiuretic hormone, which appears to be increased perhaps by virtue of delayed destruction by the impaired liver function. This leads to water retention.

DR. SHERRY: After his mastectomy, the patient did well for the next six to seven years, then he began to slip and finally entered the hospital with severe gastrointestinal bleeding. Mr. Sly, what do you believe was the cause of his bleeding?

MR. SLY: There are a number of things which could account for gastrointestinal hemorrhage in a patient of this sort. Alcoholics frequently have a gastritis which causes hemorrhage. We know that this patient had esophageal varices, which frequently hemorrhage. There is an increased incidence of peptic ulcer in persons with cirrhosis. In addition, prothrombin times are prolonged in some patients with cirrhosis and contribute to bleeding as does thrombocytopenia.

DR. SHERRY: What percentage of patients with cirrhosis of the liver will have severe gastrointestinal bleeding as their terminal complication?

MR. SLY: I think about 30 to 40 per cent.

DR. SHERRY: Why should individuals with cirrhosis of the liver have a high incidence of peptic ulcer?

MR. SLY: I do not know.

DR. SHERRY: A possible explanation has recently been offered. It has been suggested that when portal hypertension develops, the amines formed in the gastrointestinal tract are shunted directly to the stomach. Some of these amines, like histamine, are powerful secretagogues and may be instrumental in the development of gastric erosions and peptic ulcers. Do you believe that our patient bled from an esophageal varix or from some other site?

MR. SLY: The esophagoscopic findings do not rule out bleeding from an esophageal varix, but I think they make this less likely. I suspect it is more likely that he was bleeding from gastric varices or an ulcer.

DR. SHERRY: Mr. Kreager, does it not bother you that the hematemesis was never bright red; the blood was always altered as if it were arising from the stomach or duodenum.

MR. KREAGER: Well the patient did have a Sengstaken tube in place, and the gastric balloon and the esophageal balloon were inflated at one time, and he continued to bleed; although this does not completely rule out esophageal varices, I think that it does show that the site of bleeding is probably lower down in the gastrointestinal tract.

DR. SHERRY: I would suspect that although we have good evidence for the presence of esophageal varices, perhaps the bleeding was coming from either the stomach or a duodenal ulcer. Obviously, we cannot make a definitive diagnosis on the basis of the data available. Mr. Black, our patient had a flapping tremor at the time he entered the hospital and subsequently went into coma. Are we justified in making a diagnosis of hepatic coma?

MR. BLACK: I would say probably not, although he was possibly approaching that state.

DR. SHERRY: What criteria should be used in making a diagnosis of hepatic coma?

MR. BLACK: The patient should have hepatic disease and progress through various stages manifested by disorientation, flapping tremor and gradual deterioration of his mental status through obtundity to coma. This may be either

rapid or slowly progressive. It may be associated with precipitating situations such as hemorrhage or drugs; however it may not.

DR. SHERRY: Mr. Steiger would you be willing to diagnose hepatic coma in our patient?

MR. STEIGER: He certainly had a perfect background for it to occur. We know he had advanced liver disease and the presence of esophageal varices indicates that he had portal hypertension as well. Superimposed on this a gastrointestinal hemorrhage developed. Hepatic coma is often precipitated by such a hemorrhage. It has been suggested that the shock associated with hemorrhage might precipitate necrosis of the liver, and blood in the intestine of course can increase the absorption of ammonia or other possible toxins that are assumed to be responsible for hepatic coma.

DR. SHERRY: Are you satisfied on the basis of your reading that an elevated ammonia level is the cause of hepatic coma?

MR. STEIGER: No, good correlation between symptoms and blood levels of ammonia or even arteriovenous ammonia differences has been demonstrated. There have been several suggestions to explain this. For instance, it has been suggested that the concentration of ammonia in blood is not the basis of symptoms but rather the amount that can get into the brain and cerebrospinal fluid. Possibly a pH gradient between the blood and the cerebrospinal fluid might determine how much ammonia can get in. This hypothesis is based upon considerations of membrane permeability. If a membrane is permeable to the unionized species of a compound, but not permeable to the ionized species, a pH gradient across this membrane will cause a difference in distribution of the substance. It is well known that alkalosis seems to make individuals susceptible to hepatic coma, whereas acidosis seems to be both experimentally and clinically associated with increased ammonia tolerance.

DR. SHERRY: Increased ammonia levels appear to be one mechanism by which hepatic coma can be produced, but we also know that high ammonia levels can occur without coma, and that ammonia levels can be rapidly reduced without clearing of the coma. Therefore, there must be other mechanisms operative as well.

Our patient showed another interesting feature in that he became severely oliguric. Mr. Jackson, what is the mechanism of renal failure in severe hepatic disease?

MR. JACKSON: There are numerous theories, but no certain knowledge exists.

DR. SHERRY: What kinds of lesions may we see pathologically?

MR. JACKSON: Quite often the lesions are minor, but sometimes toxic lesions in the tubules are seen.

DR. SHERRY: Paper has been making a fairly extensive study of this problem. What are his thoughts concerning the renal failure which occurs during the course of hepatic disease?

MR. JACKSON: He believes that the renal failure occurs in association with a sudden lowering of the glomerular filtration rate and is due to a decrease in effective extracellular fluid volume. This, particularly when associated with hypotensive phenomena, causes the kidney to fail.

DR. SHERRY: He also has pointed out that certain events may precipitate the renal failure; one of these is gastrointestinal bleeding. Among his patients there were several who went into renal failure following gastrointestinal bleeding even though shock was not present.

Mr. Eberstein, do you think our patient had any other complications of his liver disease? Note that our patient did perfectly well until approximately one year before his death. Then he became somewhat icteric and this was followed six months later by the rapid development of ascites, portal hypertension and gastrointestinal bleeding.

MR. EBERSTEIN: No, I do not think so. This course is compatible with the end stage of cirrhosis in an individual who continues to have a high alcoholic intake and questionable nutritional status.

DR. SHERRY: Mr. Black, it is said that when a person with cirrhosis begins to get sick, suspect the presence of some new complication.

MR. BLACK: It is true that certain things will hasten the death of these patients. One is infection. The patient probably had an aspiration pneumonia.

DR. SHERRY: But he started to go downhill well before that. Did a portal thrombosis develop?

MR. BLACK: No sir. I do not think so. Portal thrombosis is often associated with pain; he did not have pain at any time that he complained of.

DR. SHERRY: But is not pain found only with an acute portal thrombosis?

MR. BLACK: Generally speaking, yes.

DR. SHERRY: Could a slowly progressive obstruction of his portal vein have developed?

MR. BLACK: I do not think you can rule this out.

DR. SHERRY: None of you appear to be attracted to the idea that a rapid rise developed in portal hypertension and that obstruction of his portal vein would explain the rapid development of ascites, edema and bleeding. Mr. Fogg, what about the possibility of a neoplasm having developed in the liver?

MR. FOGG: That certainly is a good possibility in any person who has had cirrhosis for this period of time. We cannot rule it out. It occurs in one of twenty-five patients with cirrhosis who come to autopsy. Frequently there is pain, a rapidly enlarging liver and rapid downhill course like this. There are one or two other things which can help in making the differentiation, but I do not think that we can in this case. I would be inclined to say that the patient probably did not have a primary carcinoma.

DR. SHERRY: It is interesting that in South Africa the most common neoplasm is carcinoma of the liver and that primary carcinoma of the liver occurs almost exclusively in patients with an underlying cirrhosis. Nutritional cirrhosis is quite common among the natives of South Africa. It is rather interesting that the incidence of primary carcinoma in cirrhosis is not as high in the United States or Western Europe as it is in Africa but it still represents a significant complication of hepatic cirrhosis in these former areas. I am intrigued by the fact that our patient suddenly began to do poorly, and that icterus developed as well as a rather rapid development of portal hypertension toward the latter part of his course. One could easily explain this by the development of a hepatic neoplasm with actual infiltration of the portal vein producing a partial obstruction. Did anyone else consider this possibility?

MR. SLY: I think that is a good possibility, Dr. Sherry, but I hesitate to say that he had a hepatoma at the time that his symptoms began at the age of seventy-one or seventy-two, because almost all patients die within one year of the appearance of the hepatoma.

DR. SHERRY: Your point is a good one, that when hepatomas do arise they usually run a rather rapid course. However, it is hard to date the time of development of a neoplasm. We know that from the time that symptoms first appear and the diagnosis is made, the patients usually are dead within a few months but not all patients have such a rapid course.

MR. BOREN: Would the fact that the alkaline phosphatase level was not excessively elevated, that it was out of proportion to the other liver tests, mitigate against the diagnosis?

DR. SHERRY: Alkaline phosphatase levels are usually high in carcinoma of the liver. However, we have seen patients with carcinoma of the liver without much elevation in the alkaline phosphatase level and the absence of an elevated level does not exclude a hepatic neoplasm.

Mr. Kreager, the last roentgenographic examination suggested the presence of air in the mediastinum. Is it possible that the patient's esophagus was ruptured during the course of esophagoscopy and was responsible for his terminal shock?

MR. KREAGER: I think the air in the mediastinum can be best explained by the tracheostomy tube.

DR. SHERRY: Yes, the fact that he had air in his neck and extending into the shoulders, would indicate that the tracheostomy was responsible for the mediastinal air as well.

Now we must make our final diagnosis. All of you agree that he had Laennec's cirrhosis and two of the eleven believe that the cirrhosis was complicated by a hepatic neoplasm. As for the cause of his gastrointestinal bleeding, two favor a ruptured esophageal varix, seven a hemorrhagic gastritis and two a bleeding duodenal ulcer. None believe that the kidney will reveal any specific cause for the renal failure. All of you believe that he had an aspiration pneumonia.

My own view is that the patient had Laennec's cirrhosis complicated by the development of a hepatoma with infiltration of the portal vein causing portal obstruction. As for the gastrointestinal bleeding it could be due to any of the possibilities mentioned. An aspiration pneumonia undoubtedly complicated his final course.

PATHOLOGIC DISCUSSION

DR. E. A. PORTA: The postmortem examination revealed a moderate degree of emaciation and generalized marked jaundice. The conjuctiva was covered by purulent exudate and the lens were opaque. A 3 cm. gaping tracheotomy wound was present in the neck and a sacral decubitus ulcer was also found. There was moderate edema of the ankles. The peritoneal cavity contained 3,800 ml. of straw-colored fluid; right pleural cavity 600 ml. of bloody tinged fluid and the left contained 400 ml. of a



FIG. 1. Gross picture of the liver with cirrhosis and hepatoma.

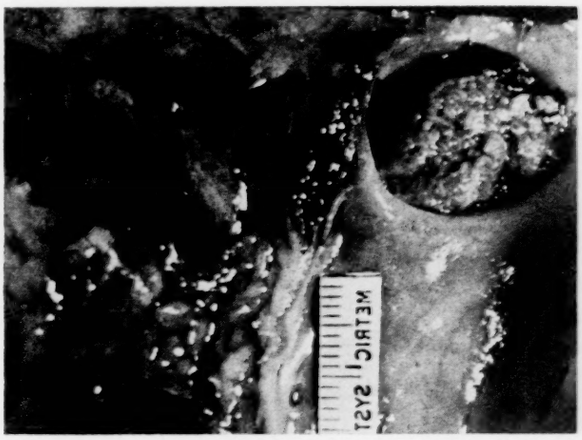


FIG. 2. Gross photograph illustrating the extension of the tumor into the hepatic vein.

clear yellowish fluid. The lungs did not collapse when the thoracic cavity was opened. They were very heavy, weighing 1,850 gm. The pleural surfaces were intact and numerous cystic dilatations and numerous rounded tumor masses could be seen through them. Trachea and bronchi were filled with mucopurulent material. A rather extensive reddened and eroded area was found in the mucosa of the anterior wall of the trachea where the cartilage was partially denuded. The cut surface of the lungs oozed a great amount of frothy serosanguineous fluid. Many areas were increased in consistency, reddish in color and in some parts, mainly in the upper lobe of the left lung, there were cavities filled with pus. Neither mediastinal nor subcutaneous emphysema was found at the time of the autopsy. The bullous emphysema of the lungs was rather prominent. Mediastinal lymph nodes were markedly enlarged and firm and the cut surface revealed replacement of the normal configuration by white and yellowish necrotic tissue. The heart appeared normal.

Microscopic examination of the non-tumorous parts of the lungs showed areas of cystic emphysema and patchy areas of bronchopneumonia with abscess formation. In addition moderate edema was found.

I should say something about the liver and about the metastatic tumors in the lungs and lymph nodes, but I would like to alter the order somewhat and start with the kidneys. Grossly they were found to be moderately reduced in size with finely granular surfaces and some scars in the cortex. Microscopically they showed arteriolosclerotic changes in moderate degree. Some tubules contained colloid casts and some were filled with small bile casts. The glomeruli showed a slight increase in cellularity but they did not show the PAS-positive thickening of the glomerular stroma commonly associated with severe cirrhosis of the liver. In the right kidney at the tip of the renal papilla there were several small abscesses containing monilia. The juxtaglomerular apparatus was rather prominent. Examination of the adrenals revealed a moderate and irregular degree of lipoid depletion. The zone glomerulosa appeared to be of normal thickness. The walls of arterioles in the periadrenal fat were markedly thickened and hyalinized. The pancreas showed numerous intralobular fat vacuoles, slight interstitial fibrosis in some areas and a moderate degree of acinar atrophy. The islets were prominent; the granularity and the proportion between alpha and beta cells was essentially normal. The spleen was firm and markedly enlarged, weighing 400 gm. The lymphoid follicles were not apparent. The red pulp was congested and many plasmocytes were found in the sinusoids. A slight increase of fibrous tissue was seen in the stroma. The liver was enlarged, weighing 1,800 gm. The enlargement was practically confined to the right lobe; the surface of which was uniformly nodular. White yellowish nodules in the upper part of the right lobe extended to the diaphragm. (Fig. 1.) On cut surface it was seen that almost the entire parenchyma of the liver had been replaced by a tremendous green tumor of soft consistency. On the posterior part of the right lobe, a white tumor mass, 5 cm. in diameter, was present which extended into one of the hepatic veins. (Fig. 2.) The only part of the liver which had

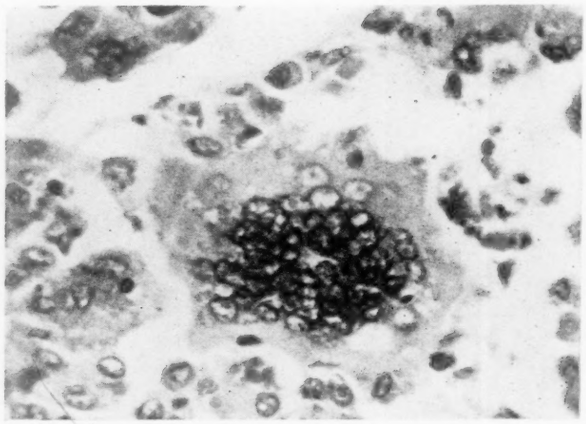


FIG. 3. High power view of a section of the hepatoma from the right lobe of the liver showing the frequently found giant cells. Hematoxylin and eosin stain.

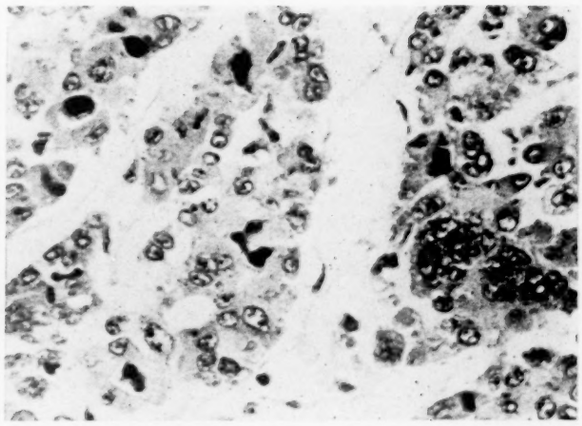


FIG. 4. Section of the hepatoma showing the pleomorphic aspect of the tumor cells in some nodules found in the right lobe of the liver. Hematoxylin and eosin stain.

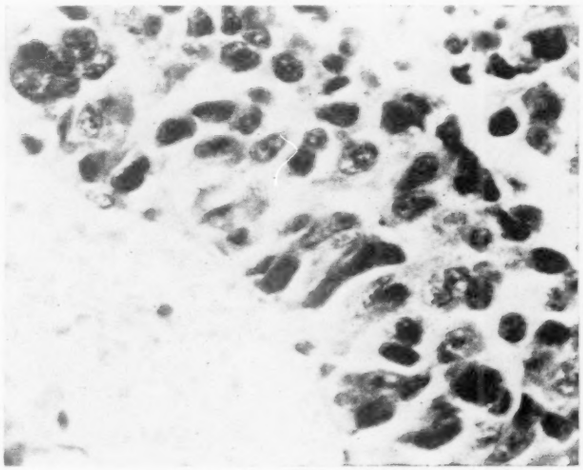


FIG. 5. Trabeculae found in some nodules formed by cells resembling the hepatic cells, but more irregular and larger. Hematoxylin and eosin stain.



FIG. 6. Electron microphotograph showing degenerating hepatic cells and a proliferating cholangiole. (From a liver lobe of the rat transplanted to the subcutaneous tissue.)

not been replaced by tumor was confined to the tip of the left lobe. In this part the parenchyma showed rounded nodules of more or less uniform size. Small greenish soft tumor metastasis were also present here. The right branch of the portal vein showed an extensive tumor thrombosis. Microscopically, one section taken from the left lobe showed a nodular parenchyma with moderate distortion of the architecture and with thin bands of connective tissue which united the portal spaces but could also be seen penetrating into the central portion of the lobules. I would prefer to call this a Laennec type of cirrhosis of moderate degree but it could possibly be a postnecrotic type. I think that cases like this, in which the fibrotic bands are not too thick, the distortion of the architecture only moderate, the regeneration marked and with some lobules apparently undamaged, could be the end result of either Laennec's or a postnecrotic type of cirrhosis. Special stains showed that this liver had practically no fat but a considerable amount of ceroid in the hepatic and Kupffer cells indicating perhaps that fat was present in some stage of the process. A small amount of iron was found in the bands of connective tissue. In the right lobe of the liver there were several tumor nodules, some composed mainly of multinucleated cells (Fig. 3) while others were composed of trabeculae which were formed by cells resembling hepatocytes with numerous bile plugs in the cytoplasm. (Fig. 4.) One small

branch of the portal vein in this lobe was seen to be occluded by a tumor thrombus. The nodular right lobe of the liver was composed of many nodules containing mainly multinucleated giant cells with bile plugs in their cytoplasm and some nodules formed by thick trabeculae of cells resembling the hepatic cells but more irregular and larger. (Fig. 5.) Areas of necrosis predominate in the latter. The yellowish white nodule seen grossly in the right lobule extending to the hepatic vein was mainly formed by highly necrotic tumor tissue of the trabecular type. Metastases found in the porta hepatis, mediastinal and mesenteric lymph nodes as well as in the lungs and the diaphragm were composed of the trabecular type of tumor cells without any evidence of bile production. In the right lobe of the liver between nodules were compressed strands of more normal appearing liver cells and a proliferation of the cholangioles. It is thought by some that in conditions similar to this, cholangioles are derived from hepatic cells. There is not satisfactory evidence for this concept. In some of our electron microscopic studies of transplanted liver of the rat to the subcutaneous tissue in which compression of tissue and impairment of bile and blood flow are important features, the hepatic cells degenerate and die and cholangioles proliferate from the remaining cholangioles. (Fig. 6.) Finally, I must say that our patient had esophageal varices which were not associated with ulceration of the mucosa. The gastrointestinal tract was within normal limits.

The final anatomic diagnoses were: Cirrhosis of the liver (long-standing; type indeterminate); hepatoma with multinucleated giant cells and with bile formation; invasion of left hepatic vein by tumor; tumor thrombus in the right branch of the portal vein with necrosis in the right lobe and marked jaundice; metastasis to diaphragm, lungs and porta hepatis, peripancreatic, retroperitoneal, mediastinal, intertracheobronchial and laterotracheal lymph nodes; jaundice, severe; ascites 3,800 ml.; pleural effusion bilateral; esophageal varices; splenomegaly; atrophy of the testes; cystic emphysema of the lungs; confluent bronchopneumonia, with mucopurulent tracheobronchitis; abscess formation in the upper lobe of the left lung; edema of the lungs; focal hemorrhagic cystitis; and monilia in renal papillae.

Case Reports

Studies on Renal Enzymes in a Patient with Renal Tubular Acidosis*

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In the twenty-four years since the first descriptions of renal tubular acidosis by Lightwood [*1*] and Butler [*2*] there has been no adequate explanation of the etiology or patho-physiology of this disorder. The essential clinical feature is the failure of the kidney to form acid urine in the presence of systemic acidosis without glomerular insufficiency. Several hypotheses based upon data obtained from renal clearance studies have been proposed to explain the defect in this syndrome. Albright [*3*] originally observed insufficient formation of ammonia following an acid load. Latner and Burnard [*4*] demonstrated normal production of acid and ammonia during infusion of neutral phosphate buffer and suggested a defect in proximal tubular reabsorption of bicarbonate as the underlying disorder. More recently, Smith and Schreiner [*5*], and Reynolds and Martin [*6*] have shown that bicarbonate reabsorption is quantitatively intact. The present study is an attempt to resolve these opposing observations by an examination of the basic intracellular enzymatic processes concerned with the production of hydrogen ion and the metabolic generation of energy.

METHODS

The patient, a four year old girl, was hospitalized in the Metabolic Unit of the Children's Hospital throughout the period of investigation. Urine was collected under oil after direct voiding and immediately refrigerated. All serum analyses were performed on blood drawn from an antecubital vein and collected under oil. During the balance studies, intake was calculated from the analysis of duplicate and refusal diets. The details of the chemical analyses have been previously described [*7*].

Assays of enzymatic activity were carried out as follows: carbonic anhydrase by a modification of the method of Krebs and Roughton [*8*]; glutaminase by the technic of Rector et al. [*9*]; diphosphopyridine nucleotide (DPN) diaphorase according to the method of Mahler [*10*]; triphosphopyridine nucleotide (TPN) diaphorase by a modification of the DPN diaphorase method, replacing the alcohol with glucose-6-phosphate, the alcohol dehydrogenase with glucose-6-phosphate dehydrogenase and the DPN by TPN; the reaction sequence from citrate to α-ketoglutarate by measuring the reduction of TPN at 340 mμ. in a recording photometer with citrate as substrate. Enzyme activity was demonstrated by histochemical technics according to the methods of Gomori [*11*] and Farber et al. [*12*].

CASE REPORT

A girl, three years and ten months of age, was first admitted to the Children's Hospital in May 1958 with bilateral congenital dislocation of hips and bilateral renal calculi. The prenatal course was uneventful and the weight at birth was 3.4 kg. The dietary intake was normal. During the first three years of life, the patient was hospitalized on four occasions for attempted correction of the dislocated hips. Several urine specimens obtained during her first admission at ten months of age showed an alkaline reaction. A right renal calculus was first noted at the age of two years three months. Because of the appearance of bilateral ureteral calculi in April 1958 she was referred to the Children's Hospital. There was no suggestion of pyelonephritis in the past history. Family history did not reveal any renal disease or congenital anomalies. The pH of the urine of both parents and two siblings ranged from 4.8 to 5.3.

The patient weighed 15.6 kg. and was 96 cm. tall. Blood pressure was 104/60 mm. Hg. The only abnormal physical findings were bilateral dislocation

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of the hips and bilateral metatarsus varus. The initial laboratory studies gave the following results: hemoglobin, 9.6 gm./100 ml.; urine, specific gravity 1.019; pH 7. Occasional red blood cells were present in the microscopic sediment. Serum analyses showed hyperchloremic acidosis, with a pH of 7.35; carbon dioxide content, 16.8 mEq./L; sodium, 143 mEq./L.; potassium, 4.9 mEq./L.; calcium, 9 mg./100 ml.; phosphorus, 4.5 mg./100 ml.; alkaline phosphatase, 9.5 Bodansky units; and non-protein nitrogen, 34 mg./100 ml. Roentgenograms demonstrated generalized undermineralization of the bones studied. The patient underwent bilateral lithotomy. Chemical analysis of the stones showed that they were composed of calcium and phosphorus.

The patient was readmitted to the hospital in October 1958 at the age of four years and 3 months. The results of the physical examination at this time were essentially unchanged from the previous admission. The weight was 15.5 kg.; height was 98 cm.; surface area was 0.65 sq. M. The urine pH continued to be neutral or alkaline. The systemic acidosis had improved, with a serum pH of 7.43 and carbon dioxide content of 24.2 mEq./L. The patient was transferred to the Metabolic Unit for investigation.

RESULTS

Metabolic Studies. While on an acid-ash diet containing 15 to 20 mEq. excess per day of inorganic anion, the urine remained alkaline. The glomerular filtration rate, estimated by endogenous creatinine clearance, was normal (57 to 60 ml./minute/sq. M.). Urine cultures were sterile. The urinary amino acid excretion (42 to 47 mg./day, expressed as α-amino nitrogen) was not excessive, and the pattern of amino acids studied by paper chromatography was qualitatively normal. The daily output of citric acid in the urine (46 to 64 mg.) was about 50 per cent of the normal amount for this age (100 to 150 mg.). Unlike most adults with this syndrome [*3,5*], the patient exhibited no tubular concentrating defect, the specific gravity of the urine rising to 1.025 after overnight water deprivation. The defective reabsorption of water exhibited by these patients usually does not occur until calcium has been deposited within the kidney, suggesting that the hyposthenuria is secondary to the nephrocalcinosis. (*cf.* Albright's Cases 3 and 4.)

Response to ammonium chloride: After three days of control observation the patient was given 100 mEq./sq. M./day ammonium chloride solution. The four-day period of acid loading was well tolerated clinically with only slight diminu-

TABLE I

METABOLIC DATA DURING ADMINISTRATION OF AMMONIUM CHLORIDE

Period	Urine Excretion per Twenty-Four Hours (mM/L.)												Serum (mM/L.)				
	Urine pH	Carbon Dioxide	Titratable Acidity	Organic Acids	Ammonium	Sodium	Potassium	Chloride	Calcium	Phosphorus	Magnesium	Serum pH	Carbon Dioxide	Sodium	Potassium	Chloride
Control																
	7.16	7.4	2.0	15.0	20.1	55.7	15.3	45.5	1.1	7.3	1.0	7.43	24.0	141.0	4.4	109.8
Ammonium Chloride, Orally																
Day 1	6.70	3.2	6.9	21.4	35.9	83.8	23.4	110.0	3.1	11.3	2.3	...	...	...	...	...
Day 2	6.22	1.5	10.1	15.2	55.2	42.4	27.2	96.5	3.4	12.1	2.1	...	...	...	...	...
Day 3	6.26	1.5	11.7	24.2	56.7	42.0	27.0	92.8	4.1	12.0	2.8	...	...	...	...	...
Day 4	6.23	.9	10.8	16.8	50.8	41.2	21.8	88.0	4.0	11.1	1.8	7.37	17.9	141.0	3.9	115.4

TABLE II
BALANCE DATA

Data	Totals for the Four-Day Period											
	Sodium (mM)	Potassium (mM)	Chloride (mM)	Phosphorus (mM)	Calcium (mM)	Magnesium (mM)	Nitrogen (gm.)	pH	Carbon Dioxide (mEq.)	Ammonia (mEq.)	Titratable Acidity (mEq.)	Organic Acids (mEq.)
Urine	136	137	153	64	7	7	21	6.80	20	92	23	87
Stools	7	27	7	43	28	11	3	...	...	...	...	..
Intake	173	209	134	141	38	23	26	...	...	...	...	...
Balance	30	45	−26	34	3	5	2	...	...	...	...	...

tion in appetite and minimal nausea. The serum chloride rose from 110 to 115 mEq./L. and the carbon dioxide content dropped from 24 to 18 mEq./L. (Table I.) There was a slight fall in serum potassium, probably as a result of the increased urinary loss, whereas the serum sodium remained unchanged. The urinary pH did not fall below 6.22, a decidedly abnormal response. The increased excretion of ammonia only meets about 60 per cent of the chloride increment, whereas in the normal subject it should cover more than 75 per cent [*13*]. These findings are depicted graphically in Figure 1. Thus, the inability to compensate for the added intake of acid resulted in an increase in serum chloride and decrease in carbon dioxide content and pH, such as would not occur in a normal subject [*3*]. To demonstrate further the patient's limitations in maintaining a hydrogen ion gradient between urine and the renal tubular epithelium, twice the amount of ammonium chloride (200 mEq./sq. M./day) was given for three days. The minimal urine pH was 6.03, the serum carbon dioxide content 14.5 mM/L., the pH 7.30.

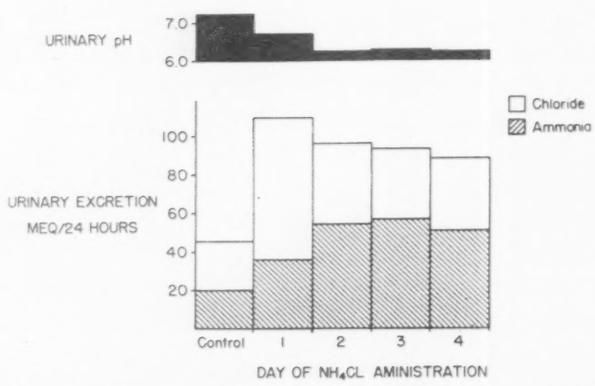


FIG. 1. Urinary response to acid loading (65 mEq. of ammonium chloride per day). The hatched areas represent that portion of urinary chloride excretion "covered" by ammonium.

Response to oral administration of sodium phosphate: Following these observations 100 mM/sq. M. of a neutral phosphate solution, pH 7.4, was given orally. The urinary excretion of phosphate increased from 8.5 to 37.4 mM daily. The urinary pH decreased from 6.56 to 6.22 [*14*], while titratable acidity increased from 5.2 to 24.8 mEq./L. There was no apparent defect in the tubular sodium-hydrogen exchange system when sufficient urinary buffer was present.

Metabolic balance studies: During a four-day control period the calcium, potassium, sodium, magnesium, phosphorus and nitrogen balance was positive. (Table II.) The negative chloride balance is difficult to interpret in such a short period [*15*]. Continued calcium loss in the urine (70 mg./day), while on a low calcium intake (400 mg./day) and with roentgenologic evidence of osteomalacia, indicated significant hypercalciuria. The positive potassium balance, which was out of proportion to the assumed tissue ratio of 3 mM. potassium to 1 gm. of nitrogen, suggests prior body depletion of potassium. Since the degree of impairment of renal ammonia production is a major factor in determining the extent of urinary cation loss, it is not surprising that this patient, with two-thirds normal output of ammonia, did not exhibit the marked potassium and calcium deficits commonly seen in adults [*3,5*].

Gastric acidity: The presence of a defect in secretion of hydrogen ion in the renal tubule prompted an examination of another site of hydrogen ion production, the gastric mucosa. Gastric juice, obtained by Levin tube after an overnight fast, had a pH of 1.61 with 24.7 units of free acid. This is within the normal range [*16*] of pH (1.6 to 1.8) and free acidity (17 to 27 units). Similar results were noted by Smith and Schreiner [*5*] in two patients with renal tubular acidosis.

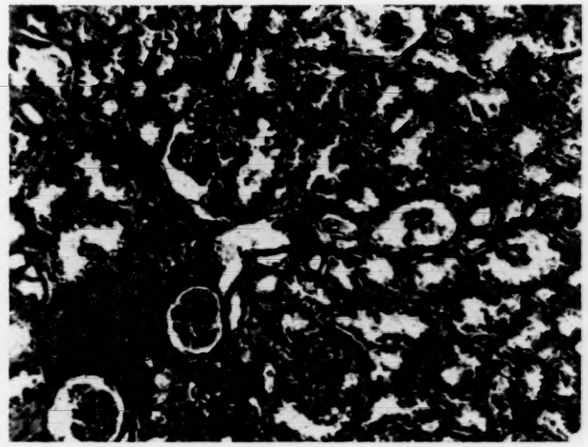


FIG. 2. Dilatation of proximal and distal tubules with periglomerular and peritubular scarring. The proximal tubular epithelium presents a rounded, mound-like luminal surface. Toluidine blue-eosin stain, original magnification × 125.

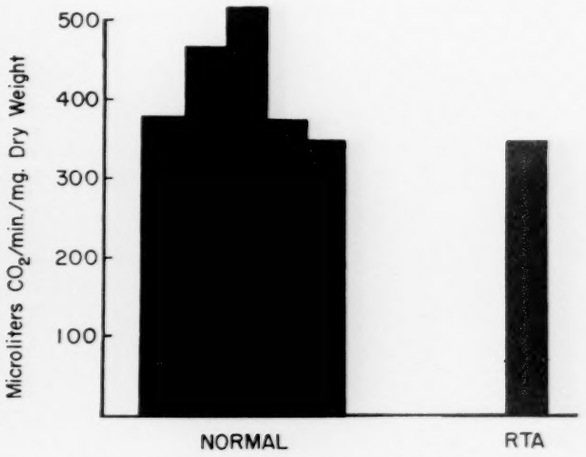


FIG. 3. Assays of carbonic anhydrase activity of kidney cortex homogenates determined manometrically at 0°C.

Carbonic anhydrase inhibition: The similarity of this syndrome to the metabolic effects following administration of acetazolamide suggested a trial of the drug to determine if inhibition of the enzyme could be demonstrated. Oral administration of a single dose of 10 mg./kg. resulted in an increase in urine pH from 7.12 to 7.72 during the four-hour period following administration of the drug. Bicarbonate excretion increased from 9.2 to 47.8 μM/minute while ammonia excretion decreased 8.9 to 3.9 μM/minute. This response indicates the presence of the enzyme, although the rate of change in bicarbonate excretion is quantitatively less than in normal subjects [*17*] and would suggest diminished enzymatic activity.

Assays of Enzymatic Activity. Six weeks following these studies a renal biopsy (surgical) specimen was obtained in order to allow enzymatic activity to return to baseline from the stimulation of an acid load [*9*]. The biopsy specimen (Fig. 2) was limited to the cortical areas in which glomeruli are evenly distributed. Most of the glomeruli showed a mild hypertrophy, with an increase in the size of the glomerular tuft. In a few of the glomerular units there was evidence of thickening of the basement membrane of the capsule although this change was rare in the glomerular tuft. A few glomeruli were completely sclerotic. The proximal tubules were moderately dilated and the epithelium was distinctive because of its rounded and mound-like free surface. There were no vacuoles in these cells and no evidence of nuclear or cytoplasmic degeneration. The mitochondria were abundant and tended to be granular in form. Although the distal tubules did not appear abnormal in cellular detail, they were dilated and contained casts of calcific material. There were scattered focal areas of tubular atrophy with an increased peritubular connective tissue. Little or no inflammation accompanied these changes, although they were associated with periglomerular scarring, suggestive of a mild healed pyelonephritis. The Sudan black stains for neutral fat were negative. The vacuolization of the proximal tubular cells and uniform thickening of the basement membrane noted in the kidneys of older patients [*18*] were not seen in this biopsy specimen, although atrophic units did show focal thickening of the basement membrane. Since the biopsy was limited to the subcapsular portion of the kidney, no loops of Henle or collecting tubules were identifiable.

Carbonic anhydrase activity: As may be seen in Figure 3 the carbonic anhydrase activity (346 μM of carbon dioxide evolved per minute per milligram dry weight of kidney cortex) was found to be within the range of the control values, 345 to 515 μM. (The control values of enzymatic activity were obtained from surgical specimens, since several of the enzymes are inactivated in postmortem material.) The decreased urinary excretion of citrate found in these patients [*19*] has been interpreted as supporting the hypothesis of diminished activity of carbonic anhydrase in this syndrome. However, the finding of normal carbonic anhydrase activity in our case would suggest that the

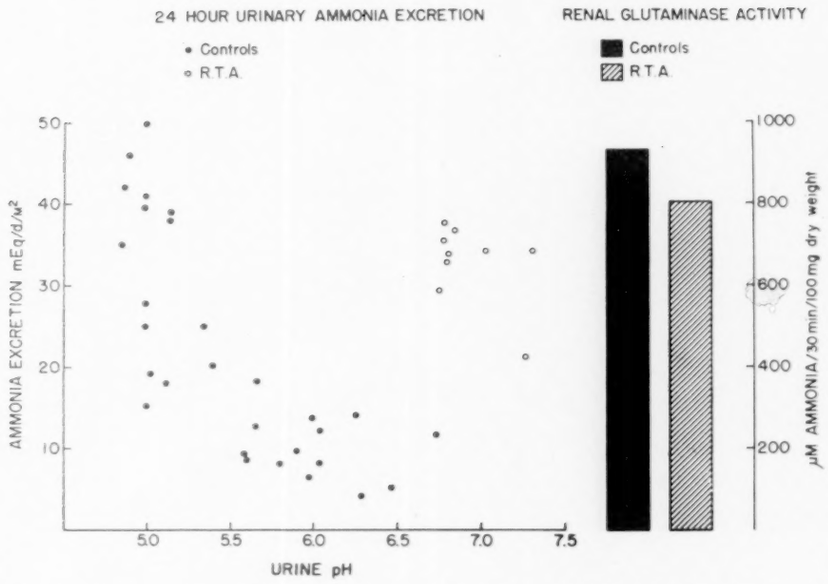


FIG. 4. Disproportion of the patient's twenty-four-hour urine pH and ammonium content as compared to control subjects. Glutaminase activities are shown at the right of the figure.

relationship between the administration of acetazolamide and decreased urinary excretion of citrate [*20*] is not directly due to inhibition of the enzyme but rather to the change in pH resulting from this inhibition.

Glutaminase activity: The excretion of ammonia in the urine is dependent upon three factors: (1) the intracellular production of ammonia; (2) the pH of the urine, which by "trapping" the weak base in the tubular lumen, governs the rate of diffusion across the tubular epithelium; and (3) the rate of urine flow, when the pH is greater than 6.25 [*21*]. A disproportionately high excretion of ammonia at the prevailing urine pH in patients with renal tubular acidosis [*19*] was also noted in this patient. (Fig. 4.) Since prolonged acidosis results in an increase in enzymatic production of ammonia [*9*], this mechanism has been suggested to explain the paradoxically increased urinary excretion of ammonia in these patients. It was surprising to find in our subject that the activity of glutaminase, which accounts for the major portion of cellular ammonia synthesis, was within the limits of the values in control subjects. Thus, no ready explanation for the discrepancy between urinary pH and increased excretion of ammonia is available.

Histochemistry: Histochemical staining technics were used in a further attempt to detect an intracellular abnormality which might be responsible for the patient's metabolic defect. In general, these methods are useful for screening purposes and where there are gross deficiencies of enzymatic activity.

The staining reactions for non-specific esterase (Fig. 5) and acid and alkaline phosphatase were normal both in intensity and in the characteristic localization within the kidney [*22*].

The Krebs tricarboxylic acid cycle is important in the production of intracellular energy and several of its key oxidative enzymes may be assayed by histochemical means. Succinic dehydrogenase (Fig. 6) and diphosphopyridine nucleotide diaphorase exhibited a staining pattern identical with that of the control. A different pattern was obtained when sodium citrate was used as the substrate for the tissue slice. (Fig. 7.) In this reaction the citrate is converted to isocitrate by aconitase present in the tissue. Isocitrate is dehydrogenated by isocitric dehydrogenase with reduction of TPN to TPNH. In the histochemical reaction the TPNH presumably passes hydrogen ion to a flavoprotein, TPN diaphorase, which reduces the tetrazolium dye, rendering it insoluble and colored. The reaction was run on two separate occasions with incubation times of thirty and sixty minutes against control samples of human kidney. (Fig. 8.) In each instance the control sections produced the characteristic staining reaction in the proximal convoluted tubules, whereas the

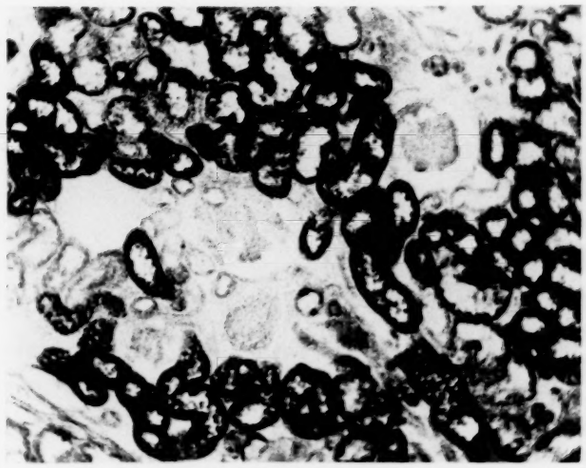


FIG. 5. Fresh frozen section of the biopsy specimen stained for esterase activity. Note intense staining in proximal convoluted tubules, with slightly less activity in the distal tubules and minimal activity in the glomeruli. Original magnification × 200.

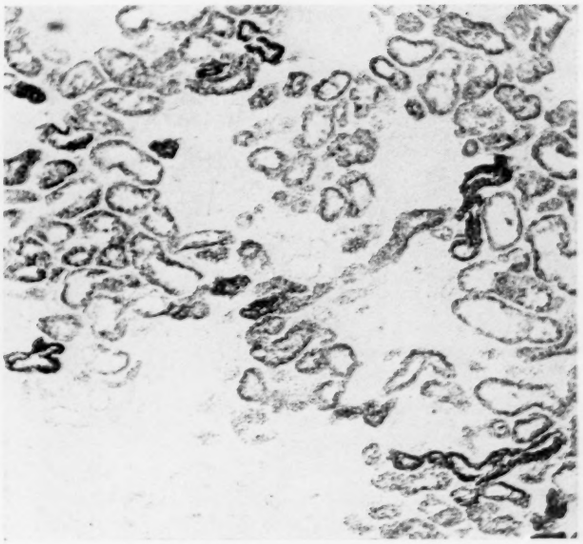


FIG. 6. Succinic dehydrogenase activity in the biopsy specimen. Enzymatic activity is confined to the tubules, with striking absence of activity in the glomeruli. Original magnification × 140.

patient's tissue caused minimal reduction of the dye.

Assay of the three enzymatic steps involved in the histochemical reaction was then performed using quantitative biochemical methods. The small amount of frozen biopsy material permitted examination only of TPN diaphorase and the reaction sequence from citrate to α-ketoglutarate. The supernatant of a 20 per cent kidney homogenate in 0.1 M phosphate buffer (which had been centrifuged at 600 × G to remove debris and cell nuclei) was utilized as the enzyme source. Specimens of normal kidneys, frozen two and six months, served as controls. The results, expressed as changes in optical density per minute per milliliter of supernatant homogenate, are given in Table III. The patient's enzyme activities were within the range of the control values, and not in accord with the histochemical results obtained previously.

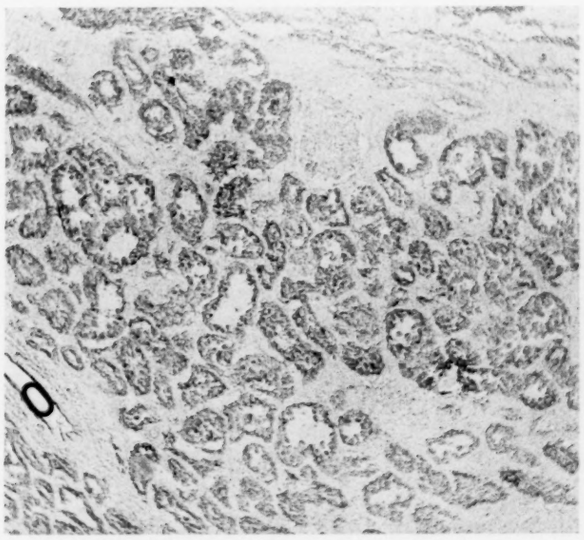


FIG. 7. TPN diaphorase activity in the biopsy specimen after incubation with sodium citrate as substrate. Note generalized decreased intensity of the staining reaction as compared to the control. (Fig. 8.) Original magnification × 125.

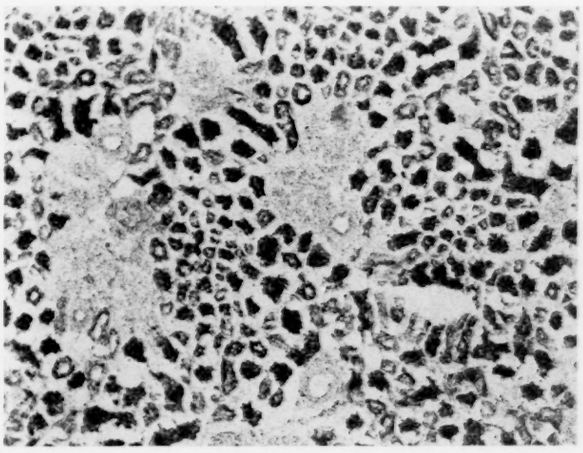


FIG. 8. TPN diaphorase activity in fresh frozen section of normal human kidney. Note intense granular staining in proximal tubules with slight activity in the glomeruli and distal tubules. There is some distortion of architecture due to the freezing technic. Original magnification × 145.

TABLE III
ASSAYS OF THE TPN DIAPHORASE-ACONITASE-ISOCITRIC DEHYDROGENASE SYSTEM
Assays made in the presence of 10^{-3} M cyanide

Subject	TPN diaphorase (Δ O.D./min./ml.)	Citrate → α-Ketoglutarate (10^3 × Δ O.D./min./ml.)
Control 1	.98	.44
Control 2	1.31	.20
Patient	1.50	.23

COMMENTS

In renal tubular acidosis the major portion of the process of urinary acidification in terms of total transfer of hydrogen ions into the urine appears to be intact. The two parameters of urinary acidification, secretion of hydrogen ion against a gradient and total transfer of hydrogen ions, may differ only in terms of energy requirements and need not imply two separate mechanisms, as suggested by others [*23*]. The inability of the patient with renal tubular acidosis to lower urinary pH with no major limitation in total hydrogen ion transfer may be due to a quantitative defect in the metabolic generation of energy.

The intrinsic factors which enable the kidney to produce urine with a hydrogen ion concentration 800 times greater than the blood are unknown. It would appear that maintenance of this steep hydrogen ion gradient across the renal tubular epithelium is an exergonic reaction. By analogy with the metabolic aspects of gastric acid secretion [*24*], these energy-producing pathways are aerobic, a good correlation existing between the secretion of acid and the consumption of oxygen. Although direct measurements of tissue oxygen uptake were not made in this patient, failure to find an enzymatic defect in the several sites of the Krebs cycle examined would suggest that perhaps another metabolic energy source is utilized in the kidney for the secretion of free hydrogen ion.

The demonstration of normal activity of carbonic anhydrase in the biopsy specimen provides conclusive evidence against a deficiency of this enzyme as the cause of renal tubular acidosis. The diminished bicarbonate diuresis (compared to normal subjects) following administration of acetazolamide is undoubtedly due to the decreased action of the drug in the presence of metabolic acidosis.

The normal activity of glutaminase does not explain the paradoxically increased amount of ammonia appearing in the urine in relation to the comparatively high urinary pH. The quantitative defect in ammonia excretion following an acid load might, therefore, be due to inadequacy of the "trapping" mechanism because of the failure to lower urinary pH.

The demonstration of an alkaline urine at an early age in this patient would favor the hypothesis that the lesion was congenital. The otherwise normal renal function would suggest that the decreased kidney function of adults with this syndrome is secondary to the calcium deposition and pyelonephritis to which these patients are prone.

SUMMARY

A metabolic case study of a four year old girl with renal tubular acidosis is presented.

Renal function was normal except for inability to maintain a hydrogen gradient across the tubular epithelium. There was minimal limitation in total transfer of hydrogen ions into the urine, as expressed by titratable acidity, ammonia and reabsorbed bicarbonate.

Histologic examination of a renal biopsy specimen disclosed dilatation of the tubules with some alteration in cellular surface. Enzyme assays for carbonic anhydrase and glutaminase activities were normal. Histochemical assay showed deficient staining for TPN diaphorase, but this was not corroborated by direct biochemical assay.

No adequate explanation for the disorder was obtained, but it is suggested that a quantitative defect in energy production might have limited the renal secretion of free hydrogen ions.

Acknowledgment: We are indebted to Dr. Duane Hagerman, Department of Biological Chemistry, Harvard Medical School, for his assistance and advice in connection with several of the enzyme assays.

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Disseminated Atheromatous Emboli

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THE occurrence of ulcerated atheroma of great vessels with intraluminal projections of lipid and fibrin masses is a common observation at necropsy. Yet, embolic phenomena from such "thrombi" apparently represent an uncommon event. Only eighty-three cases have been recorded since 1945 although brief reference to this possibility was made by Panum in 1862 [*1*]. In the majority of instances the lesions of atheromatous emboli represented incidental pathologic findings with involvement of only one or several organs, particularly the kidney, spleen or pancreas. On the other hand, such emboli have been considered responsible for myocardia infarcts [*2,3,4*], cerebral infarcts [*2,5*] and fatal renal failure [*6*], the latter occurring after operative procedures for the relief of aortic occlusion subsequent to the sequelae of atherosclerosis. Indeed, Thurlbeck and Castleman [*6*] observed atheromatous emboli in the kidneys of 77.3 per cent of patients undergoing such operations who subsequently died. Involvement of the kidney has also been implicated in the pathogenesis of some instances of hypertension by Handler [*7*], and Probstein, Joshi and Blumenthal [*8*] related these lesions to cases of acute pancreatitis. Involvement of a retinal vessel by a thin, yellow structure, observed funduscopically, and believed to be a cholesterol embolus has been noted by Witmer and Schmid [*9*]. The *intra vitam* diagnosis of this type of vascular disease has not been otherwise accomplished.

Because of the greater frequency of atherosclerotic involvement of the abdominal portion of the aorta one might expect the lower extremities to represent a common site of atheromatous emboli. However, only one documented report of such an occurrence has been recorded [*10*]. The presence of gangrene of the pedal digits has been noted by Zak and Elias [*3*] and Sayre and Campbell [*11*], but pathologic confirmation of the nature of the ischemic change was lacking.

The purpose of this report is to present the clinical and pathologic features of a patient who showed evidence of disseminated atheromatous emboli at autopsy. The clinical manifestations of his illness simulated those observed in necrotizing angiitis, notably polyarteritis nodosa. Of particular interest was the involvement of the lower extremities resulting in the clinical picture of livedo reticularis.

CASE REPORT

A fifty-two year old white man first complained of intermittent, non-radiating chest pain four years prior to death. His blood pressure at that time was noted to be 250/130 mm. Hg. Previous determinations of his blood pressure had all been within the normal range. Subsequent determinations fluctuated between 150/90 and 250/150 mm. Hg. Three years prior to death he was hospitalized for myocardial infarction and since that episode had been experiencing recurrent angina pectoris. One year prior to his death he was admitted to another hospital because of sudden severe pain in the buttocks, gastrocnemius muscles and toes. Although he experienced some immediate relief following the administration of vasodilator and anticoagulant drugs, the legs had a mottled appearance and his toes were dusky in color. Periodic pain was also experienced in the lower extremities. The patient noticed exacerbation of these phenomena during exposure to cold. Past medical and family history were non-contributory. He was admitted to the Veterans Administration Hospital, Pittsburgh, three months before his death.

Physical examination revealed his blood pressure to be 240/120 mm. Hg, pulse 82 and regular, temperature 99°F. The patient appeared well nourished but in acute distress. The toes of both feet exhibited a dusky purple color and were tender. The skin of both lower extremities extending to the pelvic brim was mottled in the characteristic fashion of livedo reticularis. Pedal pulses were palpable. The remainder of the physical examination including ophthalmoscopic examination revealed no abnormalities.

Laboratory studies disclosed a hemoglobin of 15.5 gm. per 100 ml. and hematocrit of 51 per cent. There were 9,600 leukocytes per cu. mm. with a

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FIG. 1. Cholesterol clefts in lumens of small arteries of skin beneath area of gangrene. (Original magnification × 95.)

normal differential count. Platelets were 250,000 per cu. mm. Urinalyses revealed specific gravities ranging from 1.005 to 1.024, protein 0 to 4 plus, and numerous granular casts and variable numbers of erythrocytes and leukocytes. Results of serologic tests for syphilis, cryoglobulins and cold agglutinins were negative. Tests of liver function, blood sugar, non-protein nitrogen, serum proteins, alkaline and acid phosphatases and examinations of spinal fluid were similarly within normal limits. Blood cholesterol was 225 mg. per 100 ml. with 176 mg. of esters. Lupus erythematosus preparations and blood cultures were negative. Urinary catechol amine and 5-hydroxyindole acetic acid determinations were within normal limits. Barium enema, intravenous pyelograms and skeletal survey revealed no abnormalities. A roentgenogram of the chest revealed moderate tortuosity of the aorta. Sweating was observed over the legs and metatarsals but not of the soles or toes of the feet after a sweat test with cobalt chloride and rice starch.

His hospital course was characterized by fluctuating hypertension varying from 150/70 to 240/120 mm. Hg and episodes of acute pain in the lower extremities. The latter was sudden in onset and frequently accompanied by chest pain. Electrocardiograms during such intervals often revealed atrial fibrillation and alterations of the S-T and T waves compatible with coronary insufficiency. Such episodes usually persisted for a half to one hour and required narcotics for relief of pain. Electrocardiograms were within normal limits

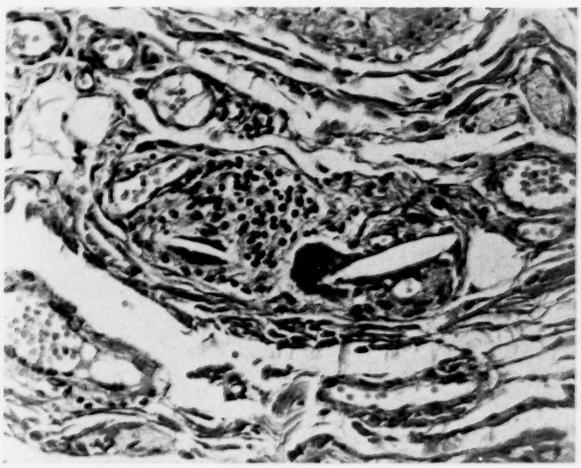


FIG. 2. Foreign body giant cell and lymphocytic infiltrate about cholesterol clefts in arterioles and small arteries. (Original magnification × 295.)

between attacks. Results of repeated regitine tests were negative. One month prior to death he had a low grade temperature averaging 100°F. accompanied by leukocytosis of 18,000 per cu. mm. He was given digitalis and maintained on a restricted sodium diet because of atrial fibrillation. Nevertheless, his course was rapidly ingravescent, gangrene of the toes ensued, and he died three months following admission to the hospital.

The findings at autopsy were as follows: petechiae were present in the skin of the chest, arms and legs. The heels and toes were purple-blue and dry. There was ulceration of the left heel and ventral aspect of the right great toe. The heart weighed 400 gm. A tan scar measuring 3 cm. was present in the myocardium of the posterior aspect of the base of the left ventricle. The coronary arteries exhibited focal occlusion by red-gray clots. The luminal surface of the aorta, extending from the arch to the bifurcation was covered by confluent, eroded, atheromatous plaques containing a superficial, villiform layer of greenish, pultaceous material. The spleen (150 gm.), liver (2,000 gm.) and kidneys (300 gm.) contained infarcts both old and recent. Inspection of the major renal arteries revealed no abnormalities.

Sections fixed in Zenker's acetic fluid, processed in the usual manner and stained with hematoxylin and eosin revealed optically clear needle-shaped clefts in the intima and/or lumina of small arteries and arterioles of the spleen, kidney, stomach, small intestine, colon, pancreas, urinary bladder, prostate, adrenals, and skin of the left heel, right thigh and quadriceps femoral muscles; the clefts being most abundant in the vessels of the spleen and kidney. In some vessels this material was principally intraluminal, causing complete or incomplete luminal obstruction. (Fig. 1.) In such instances the media of involved vessels occasionally assumed a fibrinoid appearance



FIG. 3. Intimal hyalinization about cholesterol clefts in small artery of the spleen. (Original magnification × 175.)



FIG. 4. Birefringent nature of material in clefts in lumen of the interlobular artery of kidney. (Polarized light, frozen section. Original magnification × 175.)

and often was lightly infiltrated with lymphocytes and erythrocytes. An occasional vessel contained a reaction to foreign bodies with multinucleated giant cells about such spaces. (Fig. 2.) The most frequent vascular alteration consisted of a markedly thickened, hyalinized intima encompassing cleft-like spaces. (Fig. 3.) The vascular lumina in some instances were eccentric. Only negligible perivascular sclerosis was apparent. Involved vessels frequently revealed fragmentation and apparent reduplication of internal elastic laminas in sections stained by the Verhoeff-van Gieson technic for elastic fibers. Frozen sections prepared from wet formalin fixed tissue revealed the optically clear spaces noted in routinely prepared sections to be anisotropic (Fig. 4) and vividly colored by the Schultz modification of the Liebermann-Burchard reaction for cholesterol and/or esters [*12*]. Small amounts of neutral lipid were detected in the occlusive masses in sections stained with oil red O. This material contained little fibrin as observed in sections stained by the immunohistochemical technic of Coons and Kaplan [*13*] for this protein. Preferential localization of gamma globulin was not present.

Sections of the aorta disclosed a thickened intima comprised of granular eosinophilic material containing many optically clear clefts. (Fig. 5.) Foci of intimal hemorrhage and endothelial erosion were evident. The media was thinned and contained small foci of fibrosis. Reactions identical with those observed in the vessels were noted in suitably prepared sections of the aorta. Sections of other organs confirmed the macroscopic impressions. In addition, many areas of the kidney presented a monotonous, uniform tubular appearance so that identification of various tubular segments was difficult. No alterations in the brain were noted. The occlusive masses within the coronary arteries appeared as fibrin thrombi. No cholesterol clefts could be identified within their substance.

The major anatomical diagnoses were atheromatous emboli, disseminated in the viscera, skeletal muscles and skin of the lower extremities; and thrombosis of the coronary arteries with old myocardial infarction.

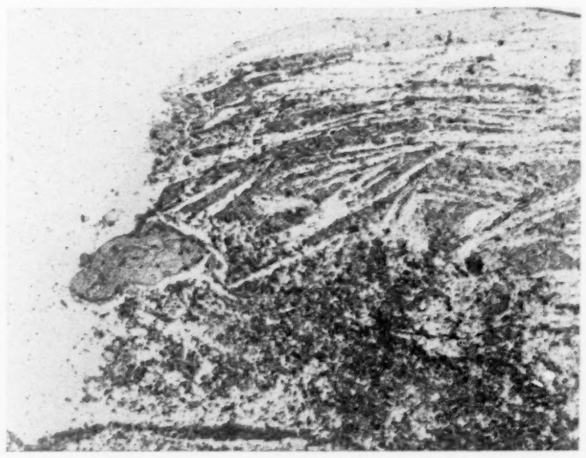


FIG. 5. Appearance of atheroma of the aorta. Numerous cholesterol clefts and hemorrhage present. (Original magnification × 115.)

COMMENTS

The embolic nature of the disseminated intravascular, occlusive masses observed in this, as well as previously described cases, appears well established. Their morphologic and histochemical identity with the atheroma of the aorta may be demonstrated by appropriate staining technics. It is germane that examples of atheromatous embolization have been noted only in association with ulcerated aortic atheroma. The failure to demonstrate incipient stages in

the development of the lesions present within small arteries and arterioles, as well as the recognition that atheroma does not represent the arteriosclerotic lesion of vessels of this caliber, militates against the possibility that the occlusive masses were primarily the result of alterations within affected vessels. In addition, the appearance of the lesions encountered was similar to those observed following the intravenous injection of atheroma in animal experiments by Flory [*14*]. Twenty-four hours after injection into a vein in the ear of a rabbit the lumen of small branches of the pulmonary artery appeared occluded by atheromatous material, erythrocytes and neutrophils. A foreign body giant cell reaction about cholesterol crystals was an outstanding feature of the vascular lesion one week following injection. The final stage was characterized by fibrosis about the cholesterol crystals, with compensation of vascular lumens. This episodic appearance of the vascular lesions was outstanding in the patient reported on herein. The presence of fibrinoid degeneration within some vessels appears to be related to the vasospasm attendant with such embolization. The production of such a lesion with epinephrine by Waters and de Suto-Nagy [*15*] tends to confirm this assumption. The phasic nature of the vascular lesions following atheromatous embolization is somewhat analogous to that noted in polyarteritis nodosa, a disease which it may clinically simulate. The morphologic appearance of the lesions in the "healed" stages in both appear similar. However, unlike polyarteritis in the healed stage the lesions of atheromatous emboli exhibit insignificant adventitial fibrosis and the vascular lumina retain an eccentric position. Careful inspection almost always reveals a cholesterol cleft in routinely prepared histologic preparations of the lesion of atheromatous emboli.

The clinical manifestations of livedo reticularis, a syndrome pathogenetically related to arteriolar spasm, in this patient appears adequately explained on the basis of the findings noted. Also, the sudden hypertension with paroxysmal fluctuations appears related to the episodic involvement of the branches of the renal artery by such emboli. The resultant ischemia and subsequent renal hypertension is also suggested by the "endocrine" appearance of the kidneys in this patient. Handler [*7*] observed hypertension and/or cardiac hypertrophy in fifteen of the twenty cases of atheromatous embolization which he reviewed, and in several this event was of abrupt onset.

Although a review of the available literature discloses a relative paucity of reported instances of atheromatous emboli arising from ulcerated atherosclerotic aortic plaques it is believed that this phenomenon is more frequent than appreciated, at least in its isolated form. In the one week subsequent to the recognition of this case, two additional examples from twenty routine, consecutive necropsies disclosed such lesions in the spleen and kidney. Although the clinical diagnosis of multiple atheromatous emboli is difficult, this disorder should be considered by the clinician in the differential diagnosis of disseminated vascular disease. Awareness of this entity by the pathologist should allow for its diagnosis in suitably selected tissue obtained at biopsy.

SUMMARY

The clinical and pathological findings of a case of disseminated atheromatous emboli occurring in a fifty-two year old white man are presented. The former were characterized by livedo reticularis of the lower extremities as well as other manifestations simulating disseminated necrotizing angiitis.

The occurrence of atheromatous emboli may be more frequent than the paucity of reported cases would indicate.

Intra vitam diagnosis of this condition appears to be dependent upon the demonstration of characteristic cholesterol crystals within vessels of suitable tissue obtained at biopsy. Such crystals may be observed principally within lumens of affected vessels, incorporated in foreign body granulomas at such sites, or within hyalinized intimal layers.

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Acute Gaseous Cholecystitis*

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Occasionally a disease entity is encountered which has such unusual characteristics that the publication of a single case is warranted. The difficulty in arriving at a rational basis for the therapy of acute gaseous cholecystitis from searching through past recorded experiences prompted this report.

CASE REPORT

A seventy-four year old white farmer (MIBH No. 11447) was admitted to the Mary Imogene Bassett Hospital on February 28, 1958, with abdominal pain of two days' duration. Ten years previously a suprapubic prostatectomy had been performed for benign prostatic hypertrophy. In the spring of 1957 severe angina, dyspnea and hoarseness developed. He had a massively enlarged heart with systolic murmurs in the mitral and aortic regions, bilateral basilar rales, bilateral pleural effusions, and an immobile left vocal cord. These signs and symptoms promptly subsided after digitalization except for mild angina, minimal cardiac enlargement and persistence of murmurs. The hoarseness cleared slowly but completely.

During the three months prior to hospital admission the patient had experienced intolerance to fatty foods and bouts of colicky pain in the right upper quadrant of the abdomen (a part of the anamnesis not elicited until the third hospital day). He had, however, been actively engaged in farm labor until he became acutely ill after supper two days before admission. At this time generalized abdominal pain developed and he vomited. He also noted the recurrence of severe hoarseness. For the next two days there were exacerbations of the pain after meals, nausea and vomiting.

On admission the patient was an elderly, acutely ill, moderately delirious, very dyspneic man whose voice was scarcely audible because of hoarseness. The pulse was 100, respirations 22, blood pressure 180/100 mm. Hg, and temperature 100.6°F. Physical examination disclosed symmetrically distributed fine, crepitant rales over the lower two-thirds of both lung fields. The heart was enlarged to the left anterior axillary line. There was an apical thrill associated with a grade 4 systolic murmur of "sea gull" quality. A grade 3 harsh systolic murmur was maximal in the aortic region and well transmitted along the carotid vessels. The cardiac rhythm was of regular sinus initiation; there was no gallop. The abdomen was somewhat protuberant, but soft. There was diffuse tenderness, more in the upper abdomen than in the lower regions. No true muscle spasm or rebound tenderness was present. The bowel sounds were normal. No abnormal masses were palpable except for easily reducible bilateral indirect inguinal hernias. The prostate was three times normal size and apparently benign. There was no jaundice.

Laboratory data were as follows: hemoglobin 14.5 gm. per cent; white blood cells 10,100 per cu. mm., with 70 per cent neutrophils; results of urinalysis were normal except for 1 plus albuminuria. Roentgenogram of the chest revealed bilateral pulmonary edema, cardiomegaly and slight elevation of the right hemidiaphragm as compared to an x-ray film taken the year before. A film of the abdomen taken on the evening of admission was entirely within normal limits.

During the first twelve hours after hospitalization the patient's condition deteriorated somewhat, with temperature elevation to 102°F. and white blood count 13,900 per cu. mm. Chest and abdominal x-ray films were repeated. After cultures of throat, sputum and blood were obtained, intramuscular tetracycline, 100 mg. every six hours, was administered. The congestive heart failure was treated with increasing dosages of digitalis, mercurial diuretics and oxygen.

Increase in diffuse abdominal pain in the right side prompted the exposure of a normal appendix under local anesthesia through a McBurney incision on the second hospital day. There was a moderate amount of peritoneal fluid which appeared serous, and a culture of this fluid was sterile. When palpated the liver edge revealed no abnormalities. Neither the gallbladder nor the kidneys were felt. The abdomen was closed without removing the appendix.

On the third day after admission the radiologist, while reviewing the abdominal film taken on the day after admission, detected two crescent-shaped gas shadows in the right upper quadrant of the abdomen which had previously escaped notice. (Fig. 1.) This gas was interpreted as being in the wall of the gallbladder. The blood culture at this time was reported positive for a gram-positive bacillus which later proved to be Clostridium perfringens by specific bacterio-

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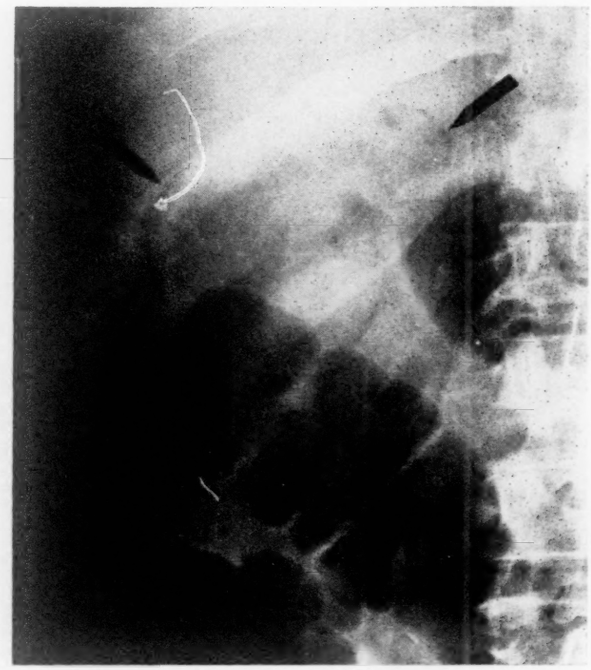


FIG. 1. Roentgenogram of the region of the gallbladder, March 1, 1958, showing two translucent crescent shaped shadows in the wall of the gallbladder. A roentgenogram on the previous day had shown no such shadows.

logical testing. It was now apparent that the patient had an acute gaseous cholecystitis with clostridium bacteremia.

Because of the patient's poor cardiac status, it was decided to treat him conservatively. On the fifth hospital day, one day after the administration of large dosages of antibiotics was started, he began to improve and continued to do so. With the exception of two brief episodes of intermittent pain in the right upper quadrant of the abdomen on the fourth and tenth hospital days, his abdominal pain and tenderness gradually diminished. Brief temperature spikes and elevations in white blood cell count coincided with these episodes. Not until after the tenth hospital day did the patient remain afebrile through a twenty-four hour period. Minimal clinical icterus was described on the second day but not confirmed by laboratory test two days later. A mass in the right upper quadrant of the abdomen became palpable on the fifth day and remained palpable for ten days.

Results of the tests of hepatic function and serum amylase levels remained within normal limits except for an abnormal bromsulphalein test on the fifth hospital day. The results of all other blood chemistry tests were normal except for a blood urea nitrogen of 26 mg. per cent on the fourth day. *In vitro* testing of the Cl. perfringens isolated from the blood showed sensitivity to penicillin, Terramycin,® tetracycline, Aureomycin,® chloramphenicol, erythromycin and Furadantin.® The organism was resistant to streptomycin and the sulfonamides.

The patient was treated for fourteen days with twenty million units of aqueous penicillin administered intravenously each day. In addition he was given 1.2 gm. of tetracycline parenterally each day during the first seven days and 3 gm. daily of oral chloramphenicol during the second seven day period of antibiotic therapy. Because the skin test was markedly reactive to gas gangrene antitoxin, this agent was not used. He was maintained on a liquid diet for the first five days; a low fat, low sodium diet was then tolerated very well. Roentgenograms of the region of the gallbladder showed increase in gas accumulation to the fifth day (Figs. 2 and 3), with gradual clearing of the gas in its lumen and wall thereafter.

At no time did he show any of the major complications of Cl. perfringens bacteremia or of the large doses of antibiotics used. The patient was discharged feeling quite well on the twentieth hospital day. He has been seen in the outpatient department on several occasions (the last time more than a year after discharge) and has been doing well.

COMMENTS

Acute gaseous cholecystitis is a pathological entity characterized by production of gas in the gallbladder during the course of an inflammatory process in that organ. Gas transported to the gallbladder via an incompetent sphincter of Oddi, a biliary gastric fistula, or a biliary intestinal fistula must be distinguished from acute gaseous cholecystitis.

The diagnosis cannot be established with certainty at autopsy because of the postmortem dissemination and gas production of clostridial organisms normally present in the intestinal tract [*1*]. Acute gaseous cholecystitis was diagnosed by chance when any surgeon during an operation for acute cholecystitis noted a distended, gas-filled gallbladder until Hegner in 1931 first made the diagnosis preoperatively by x-ray film [*2*]. The roentgenographic criteria for acute gaseous cholecystitis are: (1) an enlarged and distended gas-filled gallbladder; (2) a fluid level within the organ in the upright position; and (3) a ring of gas present in the wall of the gallbladder.

The third criterion is the most decisive, but has not been reported in all cases. Changes showing the appearance of gas in the wall of the gallbladder, the spread of this intramural gas to surrounding tissues, and later absorption of the gas are pathognomonic of this disease process.

There have been a total of seventy-three

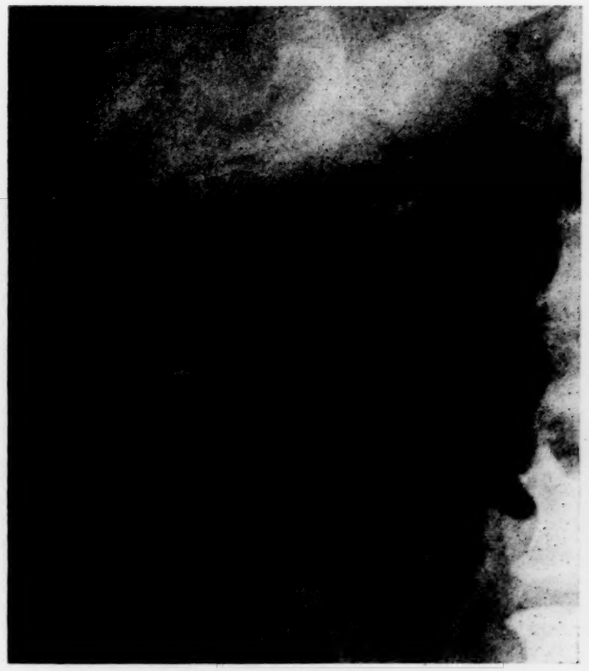


FIG. 2. Roentgenogram of the region of the gallbladder, March 3, 1958, showing the gallbladder strikingly outlined by the gas in its wall.



FIG. 3. Roentgenogram on March 4, 1958, showing increase of gas in wall and lumen of gallbladder on the fifth hospital day. Subsequent to this date there was gradual disappearance of the gas.

patients with acute gaseous cholecystitis reported on since the first report by Pende in 1907 [*3–56*]. An additional seven patients have been reported on but proof of an inflammatory process arising within the gallbladder and producing gas in that organ during the life of the patient was insufficient for acceptance as bona fide cases of the malady [*57–61*].

Men predominated in the ratio of about 2:1. The age range was seventeen to seventy-five years, sixty-two years being the median age. Twenty patients also had diabetes mellitus. In thirty-five patients previous symptomatology of the biliary tract was noted. In only eleven were the symptoms of previous disease of the gallbladder denied. (Table I.)

The onset was similar to a severe bout of acute non-gaseous cholecystitis. Usually the pain and tenderness were well localized to the right upper quadrant of the abdomen. In only one other recorded case was there noted to be such lack of localization of the abdominal discomfort as in the case herein reported. A mass was palpable on admission in twenty-four patients and became palpable in an additional seven patients later during the hospital course. Jaundice on admission was present seventeen times.

Almost half the initial temperature readings were within the normal range. Slightly over half had a temperature of 100° to 102°F., only three patients had a temperature over 102°F. when first seen. Four patients had white blood cell counts in the normal range. Most counts were in the range of 12,000 to 20,000 per cu. mm., with a high of 32,000 per cu. mm.

The type of therapy has varied. (Table II.) In forty-two cases an operation was performed within the first ten days after admission. In nine instances initial therapy was conservative, to be followed after an interval of ten to thirty days by operative intervention. In twenty-two patients the treatment was non-operative, followed after a long interval by elective cholecystectomy in four patients. Different regimens of conservative therapy have been tried. Sulfonamides, various antibiotics, polyvalent gas gangrene antitoxin, and radiation have been used. Five patients were given no specific therapy at all and did quite well. There have been six postoperative deaths [*2,11,12,20,24,25*] and one death among the patients treated conservatively [*42*].

Because of the small total number of cases and the presence of so many variables in each case, one must exercise many reservations in compar-

TABLE I
PATIENTS WITH ACUTE GASEOUS CHOLECYSTITIS

Sex	
Male	46
Female	26
Not mentioned	1
Total	73
Age (decade)	
Second	1
Third	0
Fourth	2
Fifth	11
Sixth	17
Seventh	31
Eighth	9
Not mentioned	2
Diabetes mellitus	20
Previous gallbladder symptoms	35
Denied previous gallbladder symptoms	11
Tenderness in right upper quadrant noted on admission	69
No localization to this region initially	2
Mass palpable in right upper quadrant on admission	24
Mass appeared later during hospitalization	7
Jaundice on admission	17
Jaundice developed later	3
Total with emergency and elective operations	55
Gallstones found at operation	41
No stones at operation	6
No mention of stones in operative description	8
Operative specimens examined bacteriologically	28
A clostridial organism found	16
Another organism found	8
Sterile on culture	4

TABLE II
THERAPY IN PATIENTS WITH ACUTE GASEOUS CHOLECYSTITIS

Type of Therapy	No. of Patients	Mortality (No.)
Surgical therapy		
Total number of patients operated on within ten days of admission	42	5
Cholecystectomy	12	0
Cholecystostomy	18	3
Operation not specified	12	2
Initially treated conservatively, but operated on during a ten-to-thirty-day interval after hospitalization commenced	9	1
Cholecystectomy	2	0
Cholecystostomy	3	1
Operation not specified	4	0
Total number of patients treated with surgical therapy	51	6
Conservative management		
Conservative therapy entirely	18	1
Conservatively managed, but eventually came to interval cholecystectomy after six weeks	4	0
Total with conservative therapy	22	1

ing the recorded results of the different treatment modalities. Another complicating factor in such an analysis is that since 1942 sulfonamides and since 1948 antibiotics have been used extensively in both the surgically and conservatively treated patients. However, several times a patient has been treated conservatively with an apparently good clinical result, but then the patient was operated upon because the gas did not completely disappear. Quite often this has appeared to result in increased morbidity, and even in mortality.

The association of acute gaseous cholecystitis with previous disease of the biliary tract would indicate that the pathological process involves a chronically diseased gallbladder. There has been some disagreement in regard to the pathogenic organism involved in acute gaseous cholecystitis. In many cases the patient had been undergoing antibiotic therapy for several days, and a number of different bacteriological techniques were used. Yet of the twenty-eight recorded cultures of specimens of the gallbladder removed at operation, sixteen grew one of the clostridia. (Table I.) That Cl. perfringens is present in an appreciable proportion of specimens of the gallbladder has been shown by Gordon-Taylor and Whitby (18 per cent of fifty) [*62*] and Andrews and Henry (11 per cent of ninety-one) [*63*]. An acute pyogenic infection and/or mechanically produced ischemia sets the stage by providing gangrenous tissue in which the clostridial organisms can multiply. In many of the patients a stone lodged in the cystic duct was demonstrated at operation.

The question may well be raised why active infection by the gas gangrene organism in the gallbladder is so innocuous when compared to gas gangrene of an extremity? The answer lies in anatomical differences between the gallbladder and voluntary muscle bundles of an extremity. The latter are enclosed in a dense, unyielding fascial sheath, whereas the gallbladder and neighboring organs are so invested by the areolar connective tissue that the pressure of enclosed gas is readily dissipated by dissection through contiguous fascial planes. I have repeatedly made the observation at autopsy that

if air is gently forced into the wall of the gallbladder by a syringe and fine-bored needle, the air will dissect intramurally around the gallbladder until there is one large gas-filled bulla. Then, with often very little increase of pressure, the air dissects subperitoneally along the neck of the gallbladder, along the cystic and common bile ducts, up the porta hepatis, and across the lesser and greater omenta. A large quantity of air can be injected without any apparent resistance and with scarcely any ballooning of the connective tissue structures, so widespread is its dispersion. Thus, in the gallbladder the Cl. perfringens is prevented from setting up the deadly cycle: necrosis → proliferation of organisms → elaboration of gas in a closed space → ischemia → necrosis, etc., as is the occurrence in any region of voluntary muscle.

These anatomical differences should influence the decision as to operative intervention. If gas gangrene cholecystitis is no more life threatening than any other severe case of gangrenous cholecystitis, then the patient who is a poor risk and the patient who is doing well under a conservative program should not be subjected to surgery.

It is axiomatic that cardiac decompensation (as in the patient reported on) and diabetes mellitus (often present in patients with this disease) demand careful control in the presence of a potentially overwhelming infection. Oxygen inhalation therapy is probably advisable during Cl. perfringens bacteremia, although the experimental basis for this is suggestive at best [*64*]. *In vitro* studies have indicated that a combination of penicillin with one of the tetracycline group is most effective in eradicating the Cl. perfringens [*65,66*]. The organism is also usually sensitive to chloramphenicol. The dosage should be massive to give adequate bactericidal levels in the tissues at the margin of the progressing infection. Because of the ischemic necrosis, little of the antibiotic dosage administered will arrive at the center of the infected site except by slow diffusion. A low fat diet and adequate use of antispasmodics are important components of the therapeutic program.

SUMMARY

A case of acute gaseous cholecystitis is presented. An abdominal roentgenogram and blood culture established the exact anatomical and etiological diagnosis, acute gaseous cholecystitis with Cl. perfringens bacteremia. Conservative treatment with the administration of massive doses of penicillin and parenteral tetracycline resulted in recovery.

Previous reports of this disease entity are reviewed. The pathologic disorder is described, and therapy discussed.

Acknowledgment: I am indebted to Dr. John Mithoefer, the attending physician who was responsible for directing the medical care of the patient in the case reported, and to Dr. Otto Sahler, whose roentgenological interpretations established an early diagnosis.

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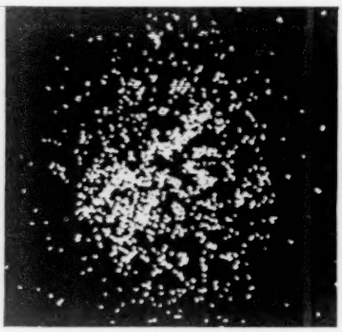
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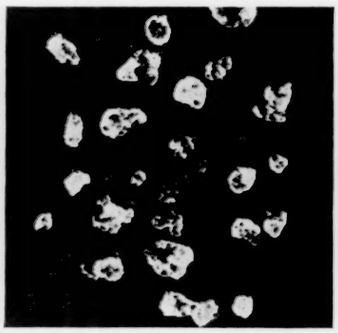
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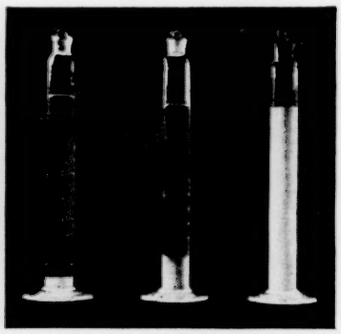
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(a) (b) (c)

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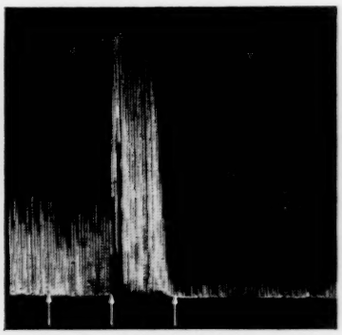
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(a) (b) (c)

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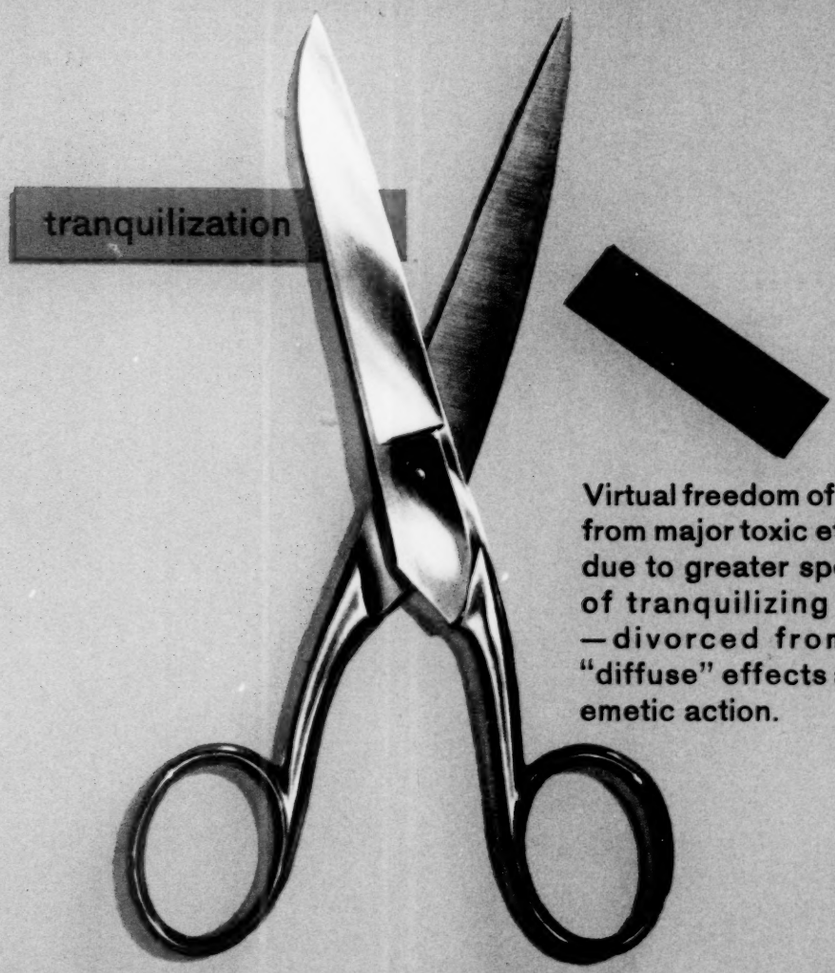
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*Kinross-Wright, J.: Newer phenothiazine drugs in treatment of nervous disorders, J.A.M.A. 170:1283, July 11, 1959.



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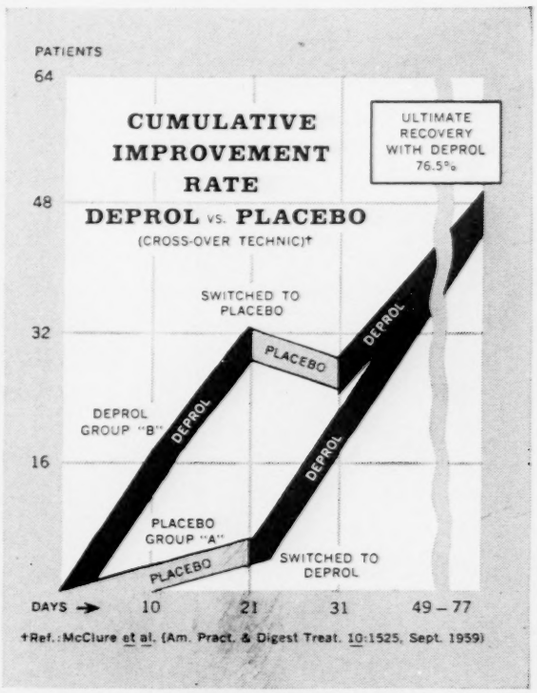
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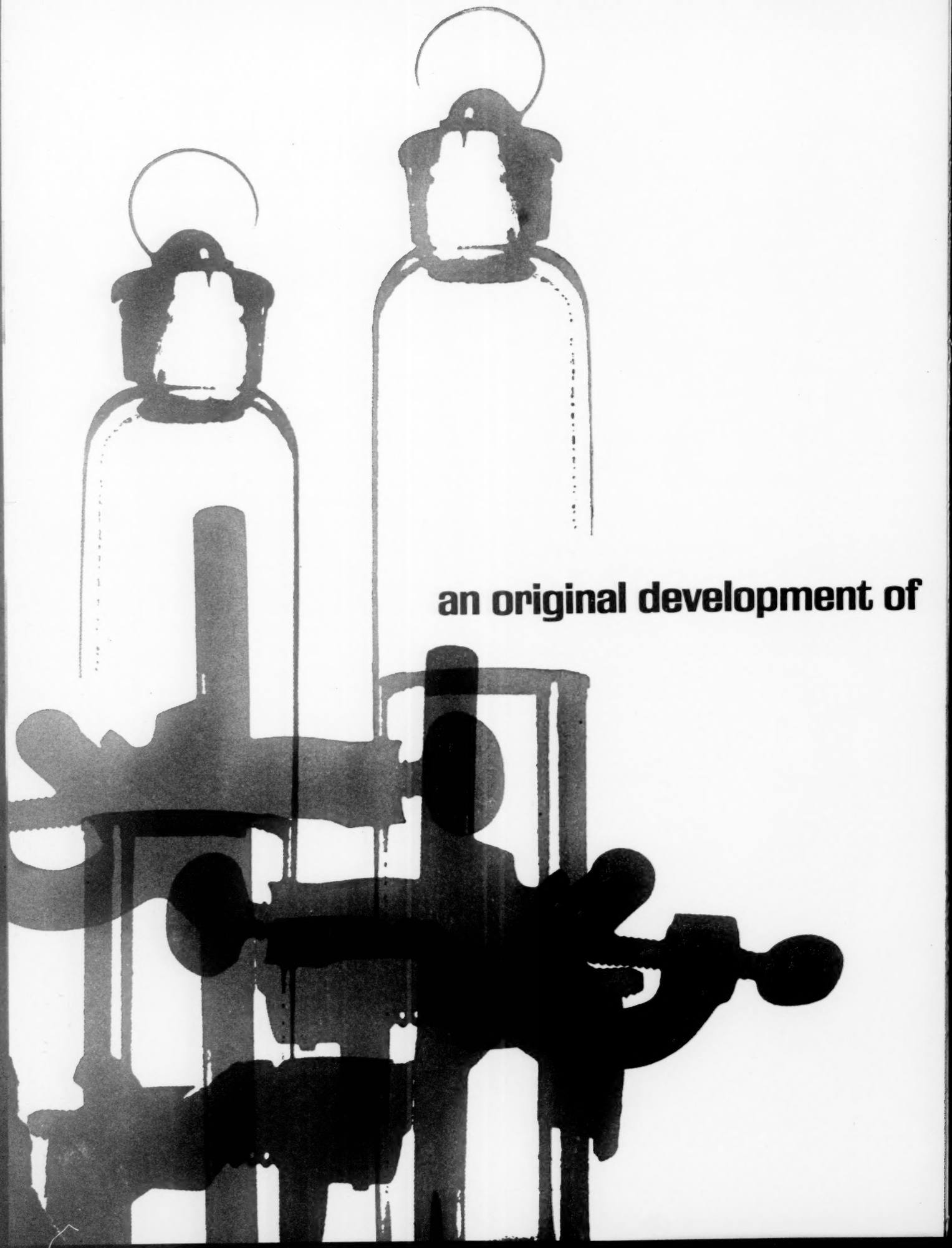
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Composition: 1 mg. 2-diethylaminoethyl benzilate hydrochloride (benactyzine HCl) and 400 mg. meprobamate.
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BIBLIOGRAPHY *(11 clinical studies, 764 patients):*

1. Alexander, L. (35 patients): Chemotherapy of depression — Use of meprobamate combined with benactyzine (2-diethylaminoethyl benzilate) hydrochloride. J.A.M.A. 166:1019, March 1, 1958. **2.** Bateman, J. C. and Carlton, H. N. (50 patients): Meprobamate and benactyzine hydrochloride (Deprol) as adjunctive therapy for patients with advanced cancer. Antibiotic Med. & Clin. Therapy 6:648, Nov. 1959. **3.** Bell, J. L., Tauber, H., Santy, A. and Pulito, F. (77 patients): Treatment of depressive states in office practice. Dis. Nerv. System 20:263, June 1959. **4.** Breitner, C. (31 patients): On mental depressions. Dis. Nerv. System 20:142, (Section Two), May 1959. **5.** Landman, M. E. (50 patients): Choosing the right drug for the patient. Submitted for publication, 1960. **6.** McClure, C. W., Papas, P. N., Speare, G. S., Palmer, E., Slattery, J. J., Konefal, S. H., Henken, B. S., Wood, C. A. and Ceresia, G. B. (128 patients): Treatment of depression—New technics and therapy. Am. Pract. & Digest Treat. 10:1525, Sept. 1959. **7.** Pennington, V. M. (135 patients): Meprobamate-benactyzine (Deprol) in the treatment of chronic brain syndrome, schizophrenia and senility. J. Am. Geriatrics Soc. 7:656, Aug. 1959. **8.** Rickels, K. and Ewing, J. H. (35 patients): Deprol in depressive conditions. Dis. Nerv. System 20:364, (Section One), Aug. 1959. **9.** Ruchwarger, A. (87 patients): Use of Deprol (meprobamate combined with benactyzine hydrochloride) in the office treatment of depression. M. Ann. District of Columbia 28:438, Aug. 1959. **10.** Settel, E. (52 patients): Treatment of depression in the elderly with a meprobamate-benactyzine hydrochloride combination (Deprol). Antibiotic Med. & Clin. Therapy 7:28, Jan. 1960. **11.** Splitter, S. R. (84 patients): The care of the anxious and the depressed. Submitted for publication, 1959.

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Published reports on Librium: 1. T. H. Harris, *Dis. Nerv. System*, *21*:(Suppl.), 3, 1960. 2. L. O. Randall, *ibid.*, p. 7. 3. J. M. Tobin, I. F. Bird and D. E. Boyle, *ibid.*, p. 11. 4. H. A. Bowes, *ibid.*, p. 20. 5. J. Kinross-Wright, I. M. Cohen and J. A. Knight, *ibid.*, p. 23. 6. H. H. Farb, *ibid.*, p. 27. 7. C. Breitner, *ibid.*, p. 31. 8. I. M. Cohen, Discussant, *ibid.*, p. 35. 9. G. A. Constant, *ibid.*, p. 37. 10. L. J. Thomas, *ibid.*, p. 40. 11. R. C. V. Robinson, *ibid.*, p. 43. 12. S. C. Kaim and I. N. Rosenstein, *ibid.*, p. 46. 13. H. E. Ticktin and J. D. Schultz, *ibid.*, p. 49. 14. J. N. Sussex, *ibid.*, p. 53. 15. I. N. Rosenstein, *ibid.*, p. 57. 16. D. C. English, *Curr. Therap. Res.*, 2:88, 1960. 17. T. H. Harris, *J.A.M.A.*, *172*:1162, 1960. 18. G. L. Usdin, *J. Louisiana M. Soc.*, *112*:142, 1960. 19. I. N. Rosenstein and C. W. Silverblatt, paper read at Pan American Medical Association, 35th Anniversary Congress, Mexico City, Mexico, May 2-11, 1960. 20. K. Rickels, *ibid.* 21. N. Toll, *Dis. Nerv. System*, *21*:264, 1960.

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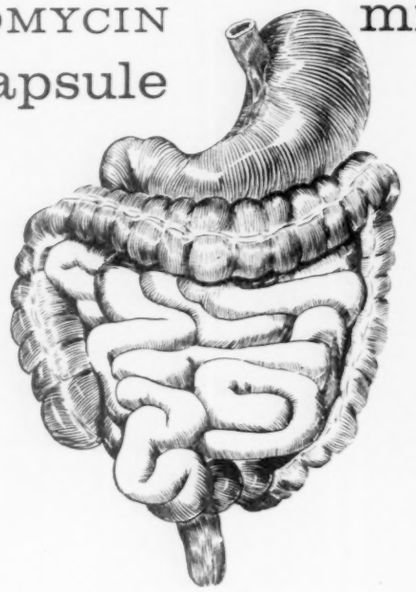
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reduction in incidence and/or severity of gastrointestinal side effects may be attributed to the far lower DECLOMYCIN milligram intake (per capsule and per day)[1-3]



1. Finland, M.; Hirsch, H. A., and Kunin, C. M.: Observations on Demethylchlortetracycline. Presented at Seventh Annual Antibiotics Symposium Washington, D. C., November 5, 1959. 2. Hirsch, H. A.; Kunin, C. M., and Finland, M.: Demethylchlortetracycline—A New and More Stable Tetracycline Antibiotic That Yields Greater and More Sustained Antibacterial Activity. München. med. Wchnschr. To be published. 3. Lichter, E. A., and Sobel, S.: The Distribution of Oral Demethylchlortetracycline in Healthy Volunteers and in Patients Under Treatment for Various Infections. To be published.

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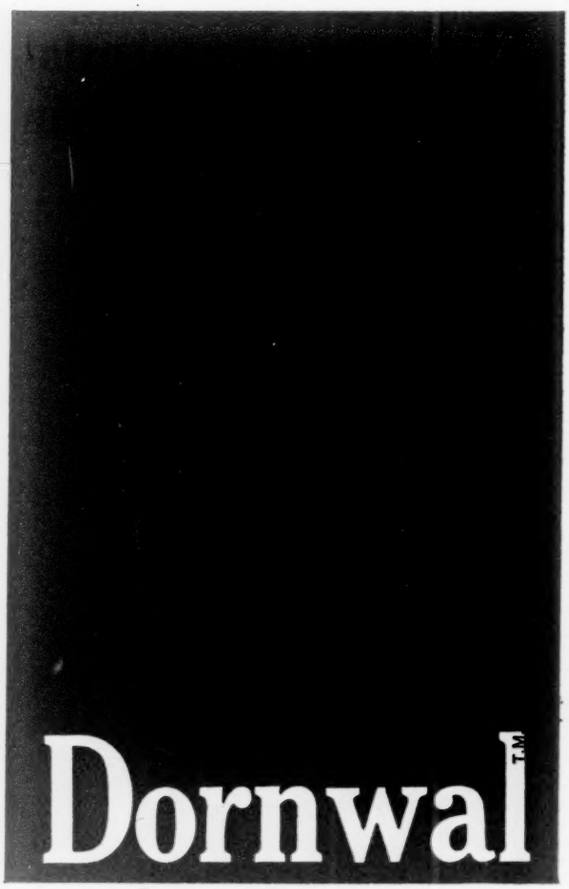
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". . . our program has been one of polypharmacy in which we attempt to deplete body sodium with chlorothiazide. This drug is continued indefinitely as background medication for all antihypertensive drugs." Moyer, J. H.: Am. J. Cardiology, **3**:199, (Feb.) 1959.



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"All three of the patients with Laennec's cirrhosis, ascites and edema had a favorable response, with a mean weight loss of 8 lbs., during the five-day treatment period with a slight decrease in edema." Castle, C. N., Conrad, J. K. and Hecht, H. H.: Arch. Int. Med., **103**:415, (March) 1959.



in renal edema

"In a study of 10 patients with the nephrotic syndrome associated with various types of renal disease, orally administered chlorothiazide was a successful, and sometimes dramatic, diuretic agent." Burch, G. E. and White, M. A., Jr.: Arch. Int. Med., **103**:369, (March) 1959.

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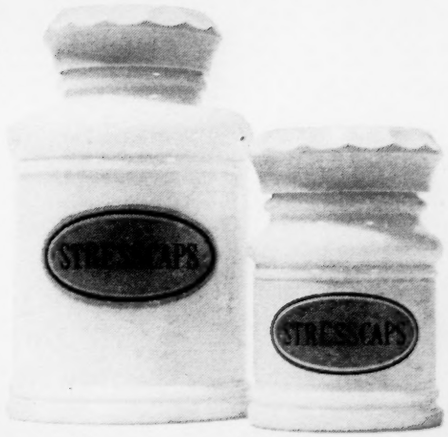
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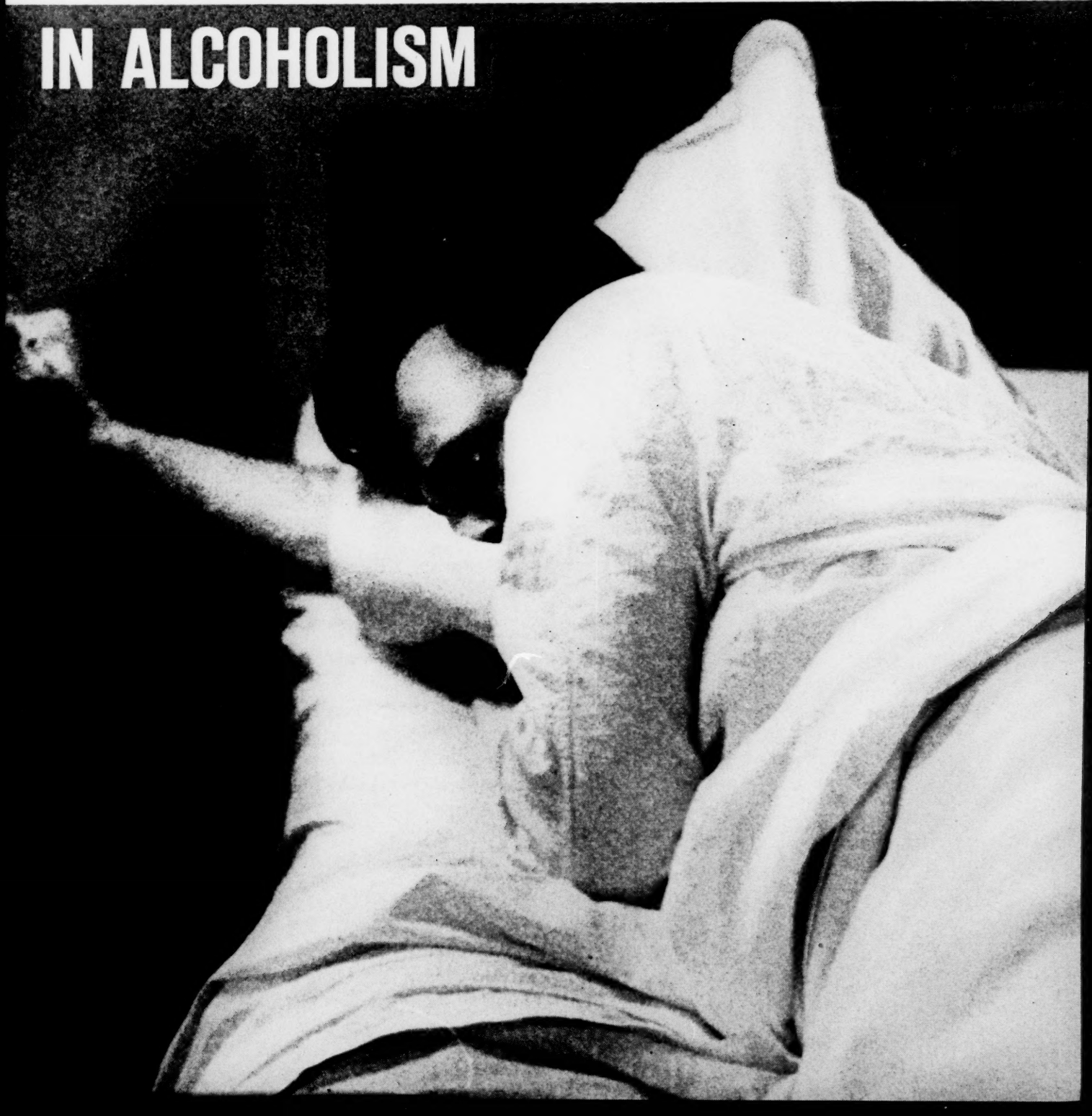


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1. Lehrer, H. W., et al.: Northwest Med. 75:1249, 1955.
2. Smith, Richard T.: New York Med. 8:16, 1952.

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References: 1. Tandowsky, R. M.: Personal communication. 2. Parsons, W. B.: *Curr. Therapeut. Res.* 2:137 (May) 1960. 3. Thompson, C. E.: Personal communication. 4. Biben, L. H.; Kurstin, W., and Protas, M.: Personal communication. 5. Hobbs, T. G.: Personal communication.

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references. (1) Antos, R. J.: Southwestern Med. *40*:695-697 (Nov.) 1959. (2) Tullis, I. F.: Initial Experience with a Simple Weight Control Formula, to be published. (3) Roberts, H. J.: Effective Long-Term Weight-Reduction—A Therapeutic Breakthrough, to be published.



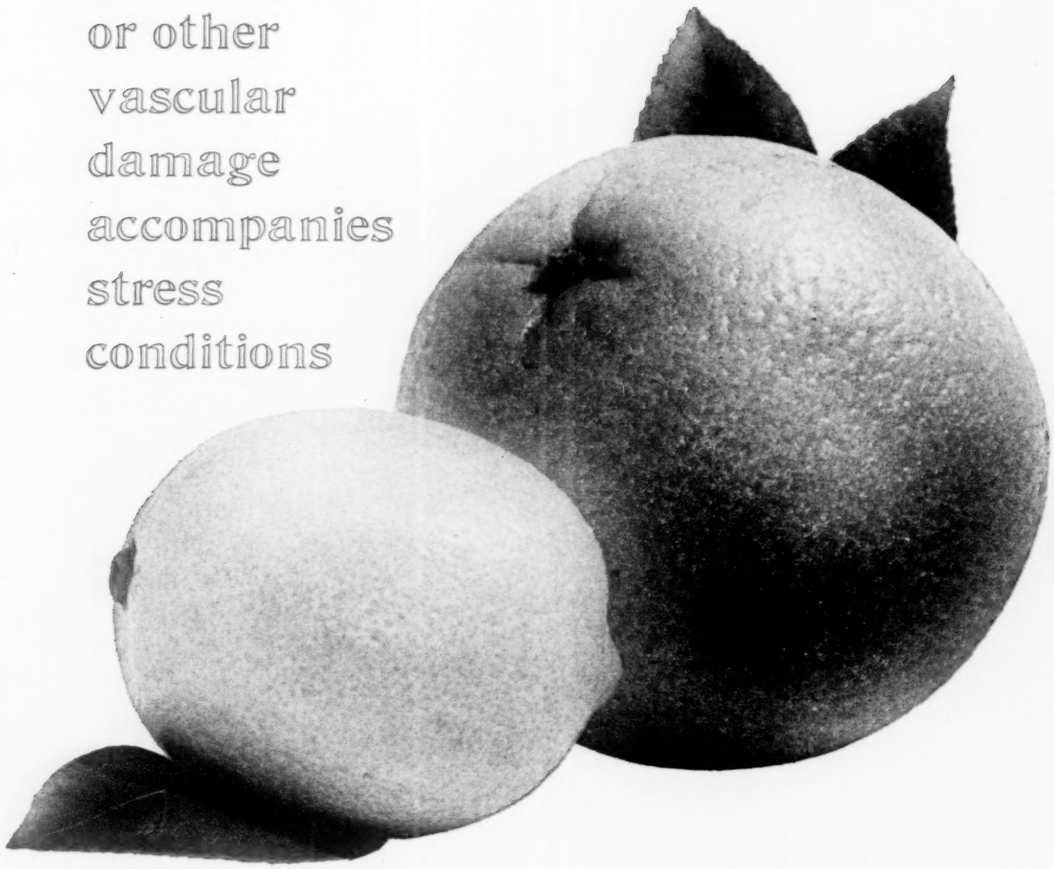
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Observations include a reduction in severity or prevention of erythroblastosis fetalis in Rh-negative patients when *Hesperidin* (7) or other citrus bioflavonoids (23, 24) were administered.

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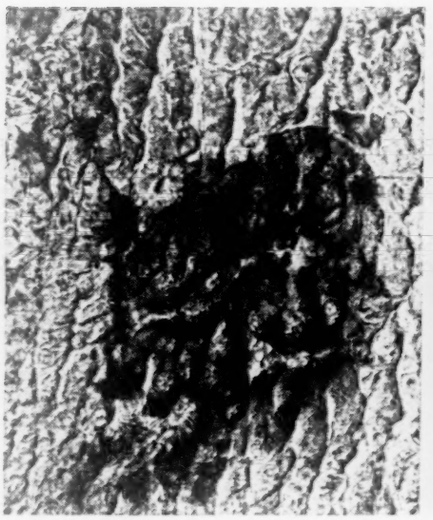
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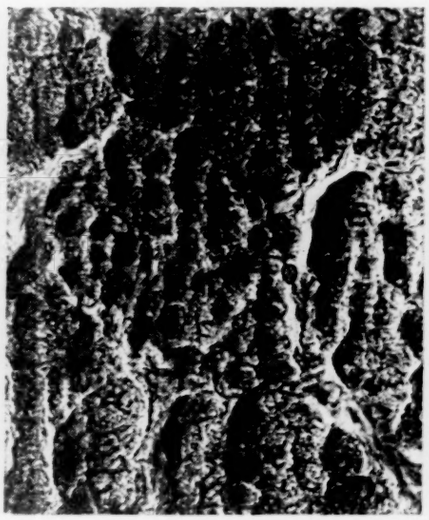
THE AMERICAN JOURNAL OF SURGERY

11 EAST 36th STREET
NEW YORK 16, NEW YORK

Prescribe enough Orinase* to release enough native insulin



Before:
Microphotograph showing insulin granules in beta cells of pancreas of normal dog.



During:
Degranulation following administration of Orinase. Note complete release of native insulin.



After:
Regeneration of granules following termination of Orinase dosage.

Adjust Orinase dosage to make available the amount of native insulin *needed* by the diabetic patient. This may be done freely because Orinase has virtually no "ceiling" imposed on dosage by toxicity or untoward effects.

In a series of 187 diabetic patients successfully managed on Orinase (tolbutamide) during a period of 6 to 30 months, the reported[1] distribution of daily doses was as follows: 1 gram, 17%; 1.5 grams, 22%; 2 grams, 40%; 3 grams, 21%.

Similarly, in three years' clinical experience with a population of approximately 3,000 diabetics on Orinase, it has been observed that about one-third of the patients at any one time require and receive dosages of 2 to 3 grams a day for successful management.[2]

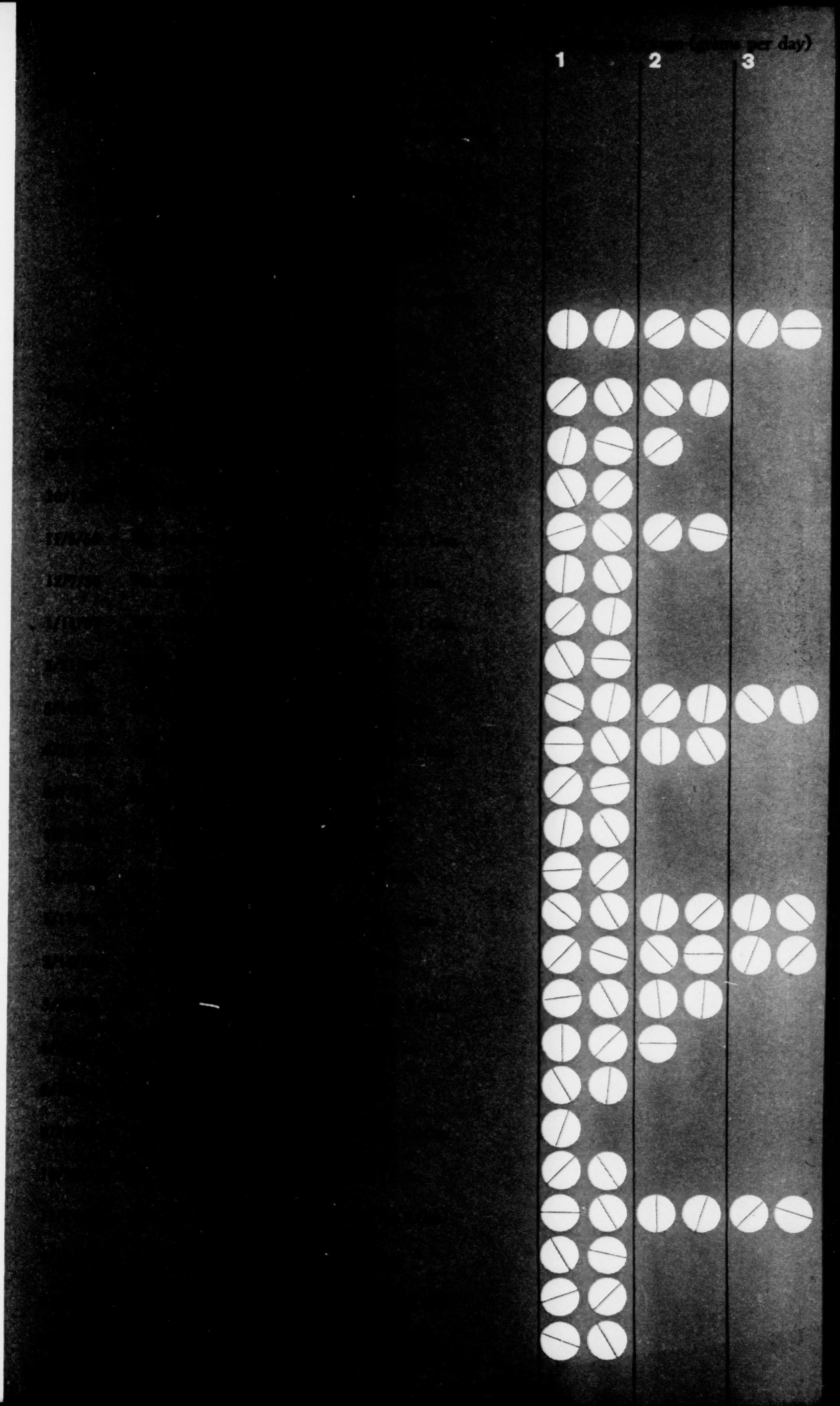
To obtain optimum control, and avoid needless "secondary failures"—give sufficient Orinase to meet varying requirements from patient to patient or in a given patient from time to time.

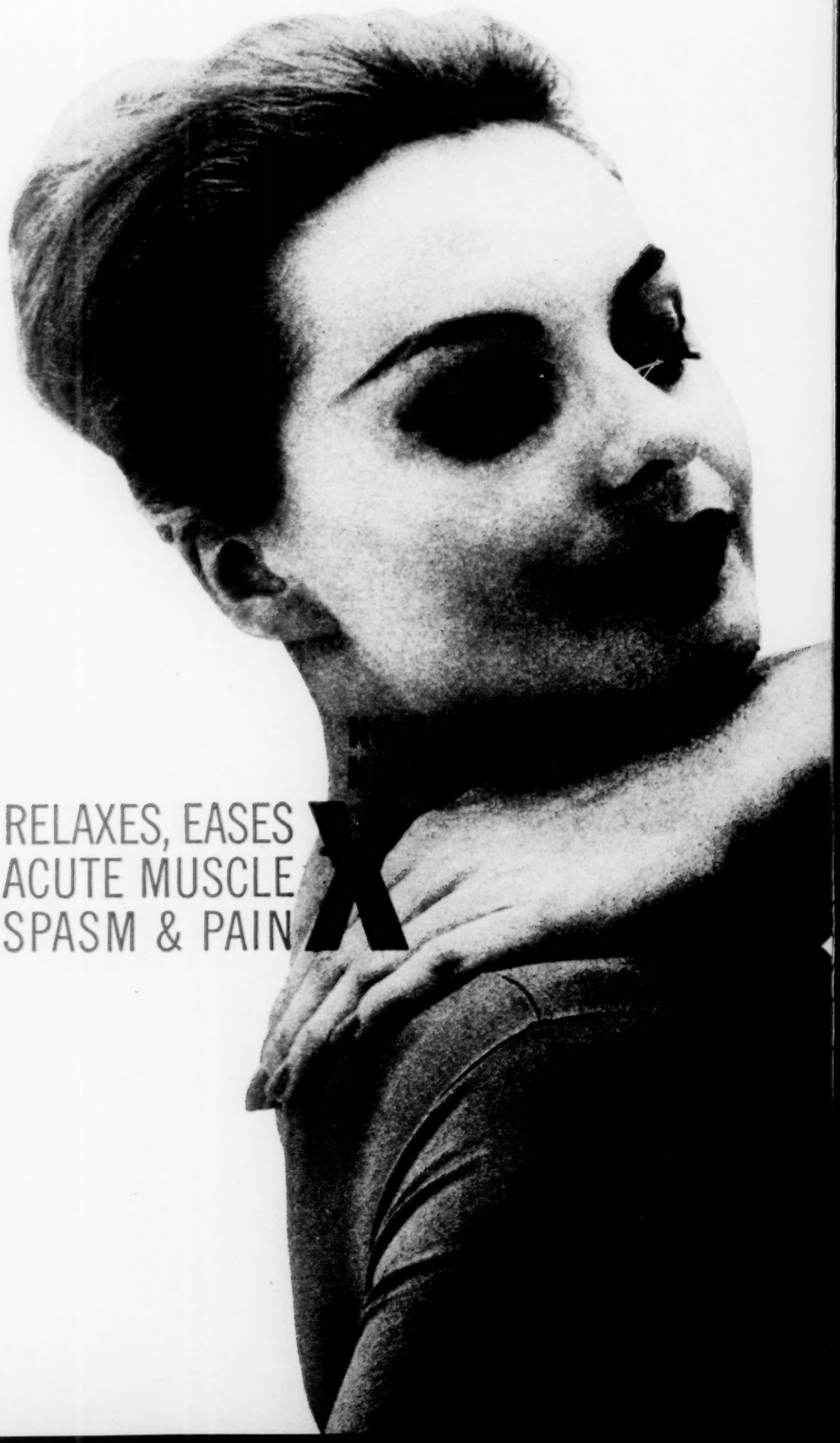
1. Gorman, C. K., and Weaver, J. A.: Brit. M. J. 2:1214 (Dec. 5) 1959.
2. Case data courtesy Henry Dolger, M.D.

*TRADEMARK, REG. U. S. PAT. OFF.—TOLBUTAMIDE, UPJOHN

Upjohn
THE UPJOHN COMPANY
KALAMAZOO, MICHIGAN

a
three-year
case
history[2]



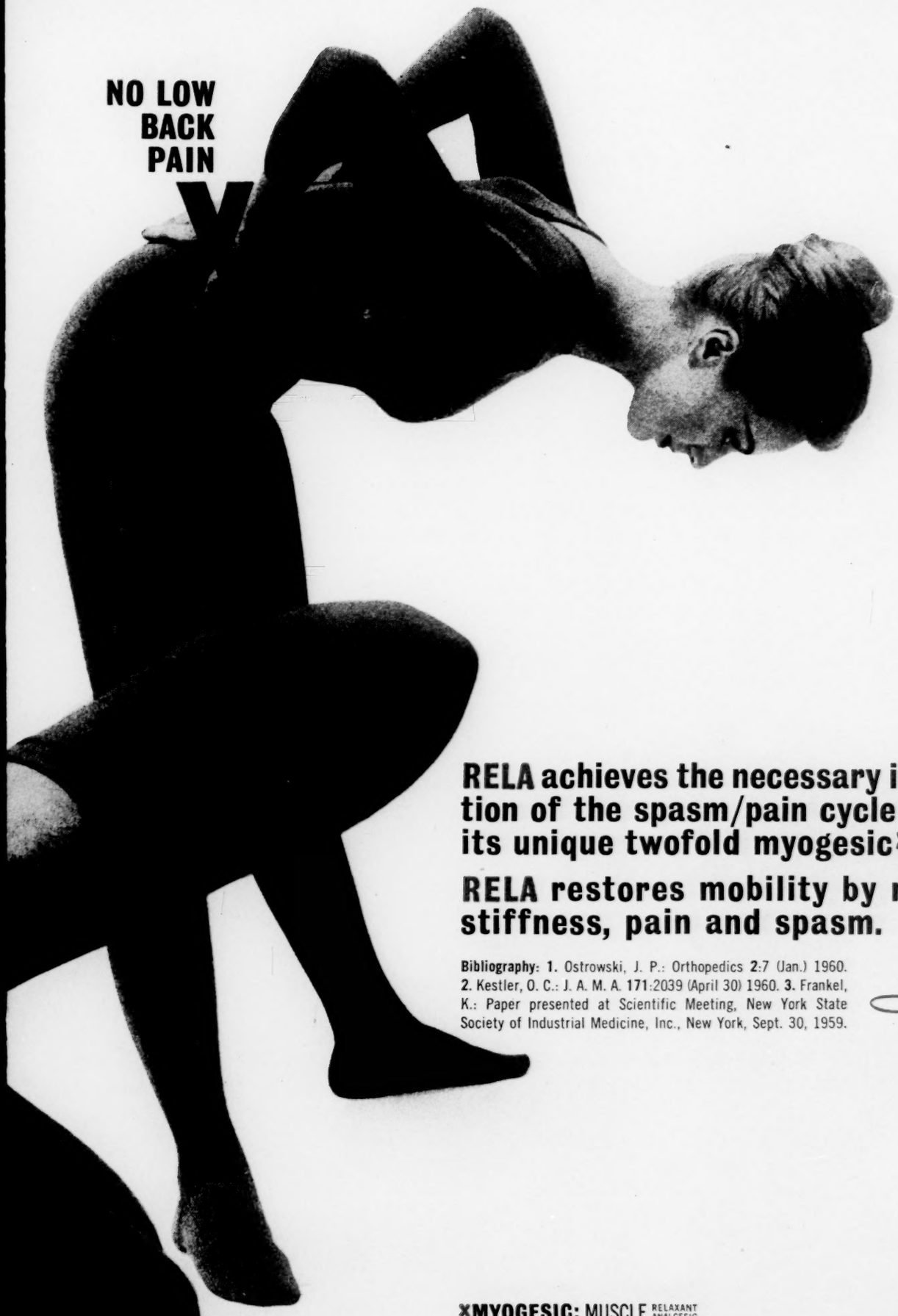


RELA™

CARISOPRODOL

RELAXES, EASES
ACUTE MUSCLE
SPASM & PAIN

M-257



NO LOW BACK PAIN

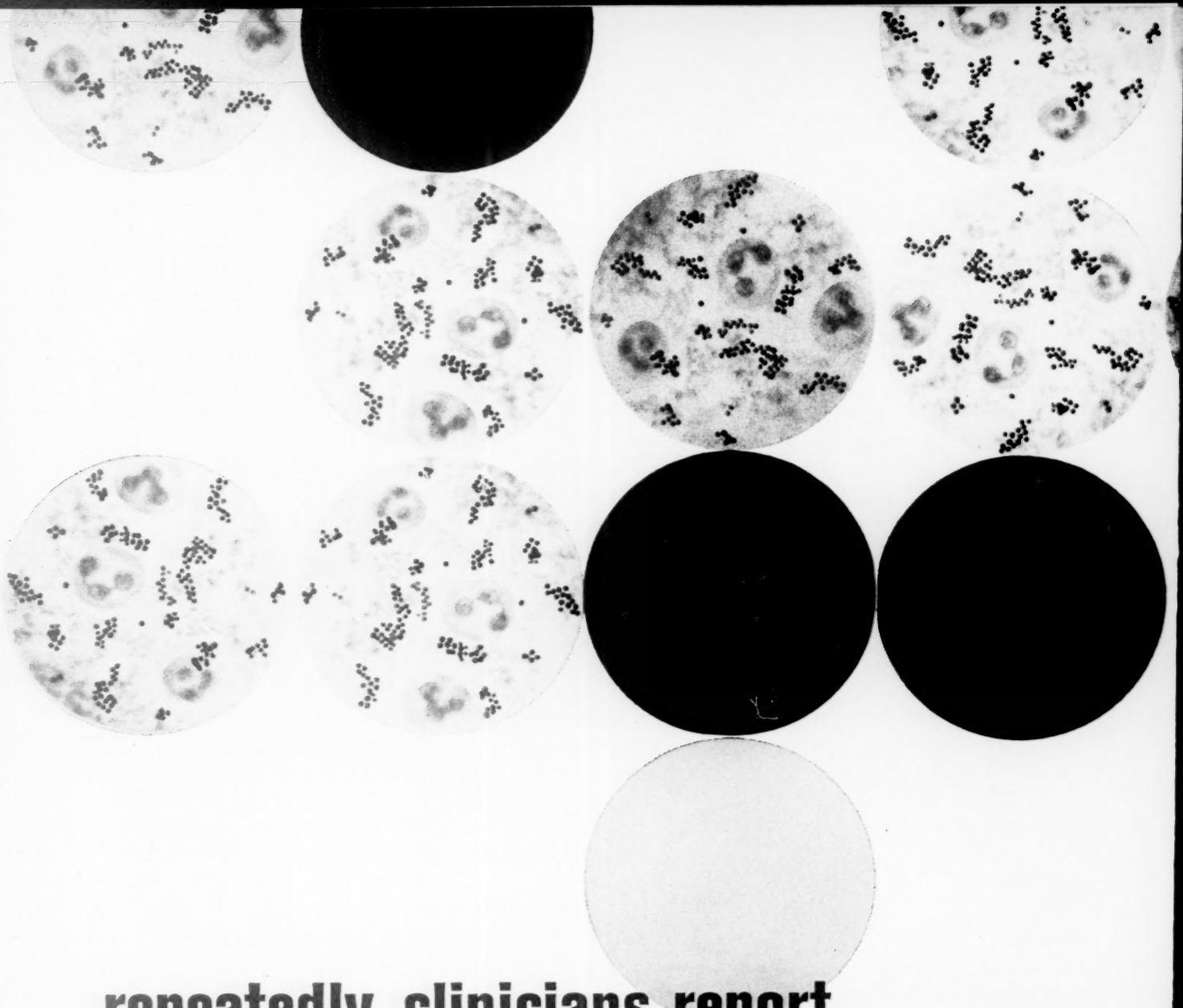
RELA achieves the necessary interruption of the spasm/pain cycle through its unique twofold myogesic[x] action.

RELA restores mobility by relieving stiffness, pain and spasm.

Bibliography: 1. Ostrowski, J. P.: Orthopedics **2**:7 (Jan.) 1960. **2.** Kestler, O. C.: J. A. M. A. **171**:2039 (April 30) 1960. **3.** Frankel, K.: Paper presented at Scientific Meeting, New York State Society of Industrial Medicine, Inc., New York, Sept. 30, 1959.

Schering

[x]**MYOGESIC:** MUSCLE RELAXANT ANALGESIC



repeatedly, clinicians report... effective results against staphylococci

"Chloramphenicol (CHLOROMYCETIN) was the only widely used antibiotic to which few of the strains were resistant."[1]

"Emergence of staphylococci resistant to chloramphenicol has rarely occurred since the development of this drug, except in an occasional clinic where it has had wide use in a closed population."[2]

"Chloramphenicol (CHLOROMYCETIN) has been an effective agent in the treatment of staphylococcal infections."[3]

"The antibiotic of choice in this series was chloramphenicol."[4]

CHLOROMYCETIN (chloramphenicol, Parke-Davis) is available in various forms, including Kapseals® of 250 mg., in bottles of 16 and 100.

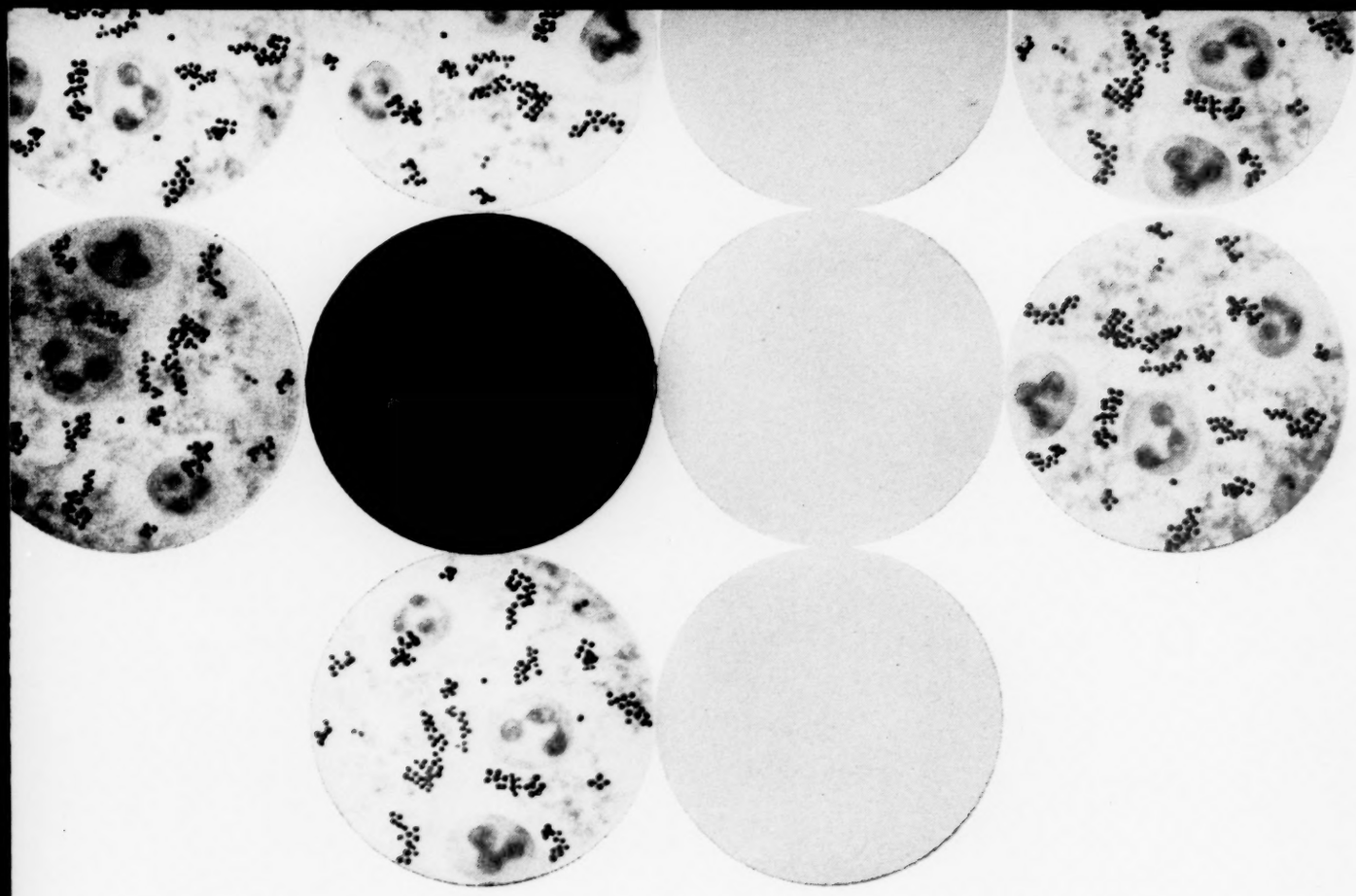
CHLOROMYCETIN is a potent therapeautic agent and, because certain blood dyscrasias have been associated with its administration, it should not be used indiscriminately or for minor infections. Furthermore, as with certain other drugs, adequate blood studies should be made when the patient requires prolonged or intermittent therapy.

References: (1) Finland, M.; Jones, W. F., Jr., & Bennett, I. L., Jr.: *Arch. Int. Med.* **104**:365, 1959. (2) Welch, H., in Welch, H., & Finland, M.: Antibiotic Therapy for Staphylococcal Diseases, New York, Medical Encyclopedia, Inc., 1959, p. 16. (3) Nichols, D. R., & Martin, W. J.: *Surg. Gynec. & Obst.* **107**:523, 1958. (4) Knight, I. C. S., & Nolan, B.: *Brit. M. J.* **1**:1224, 1959.

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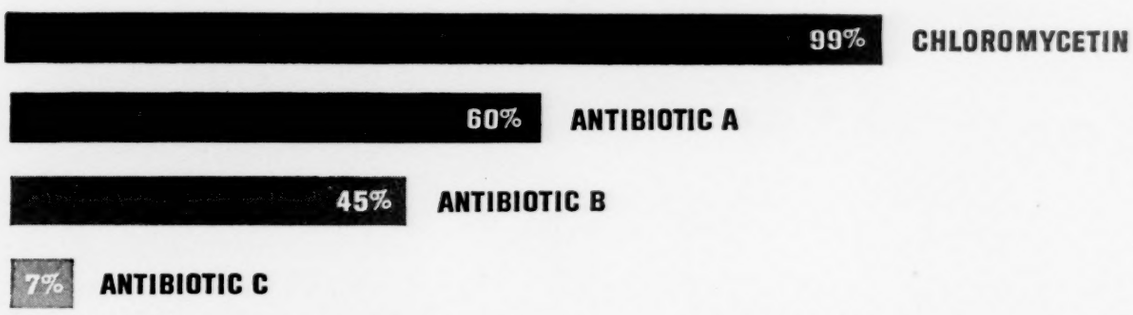
PARKE-DAVIS



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(chloramphenicol, Parke-Davis)

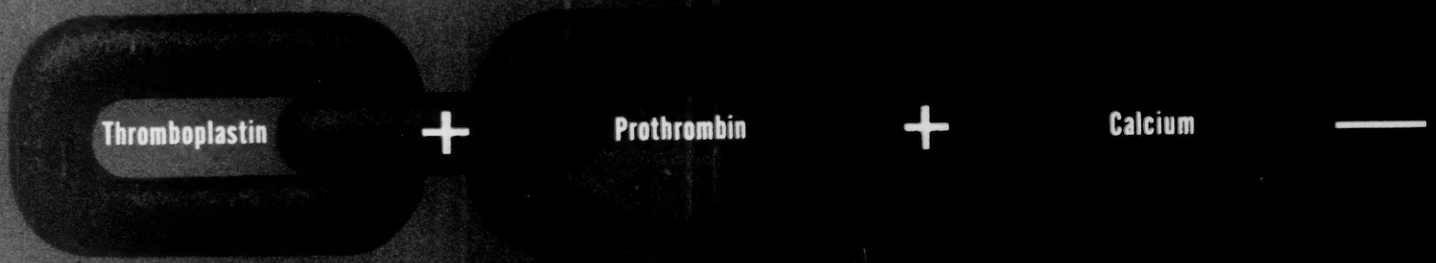
IN VITRO SENSITIVITY OF STAPHYLOCOCCUS AUREUS TO CHLOROMYCETIN AND TO THREE OTHER ANTIBIOTICS*



Staphylococcus aureus, coagulase-positive, was isolated in pure culture from 99 of 100 consecutive cases of puerperal breast abscess requiring surgical treatment.

*Adapted from Knight & Nolan[4]

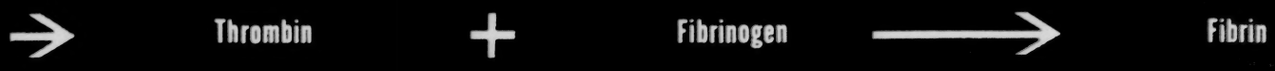
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hypoprothrombinemia



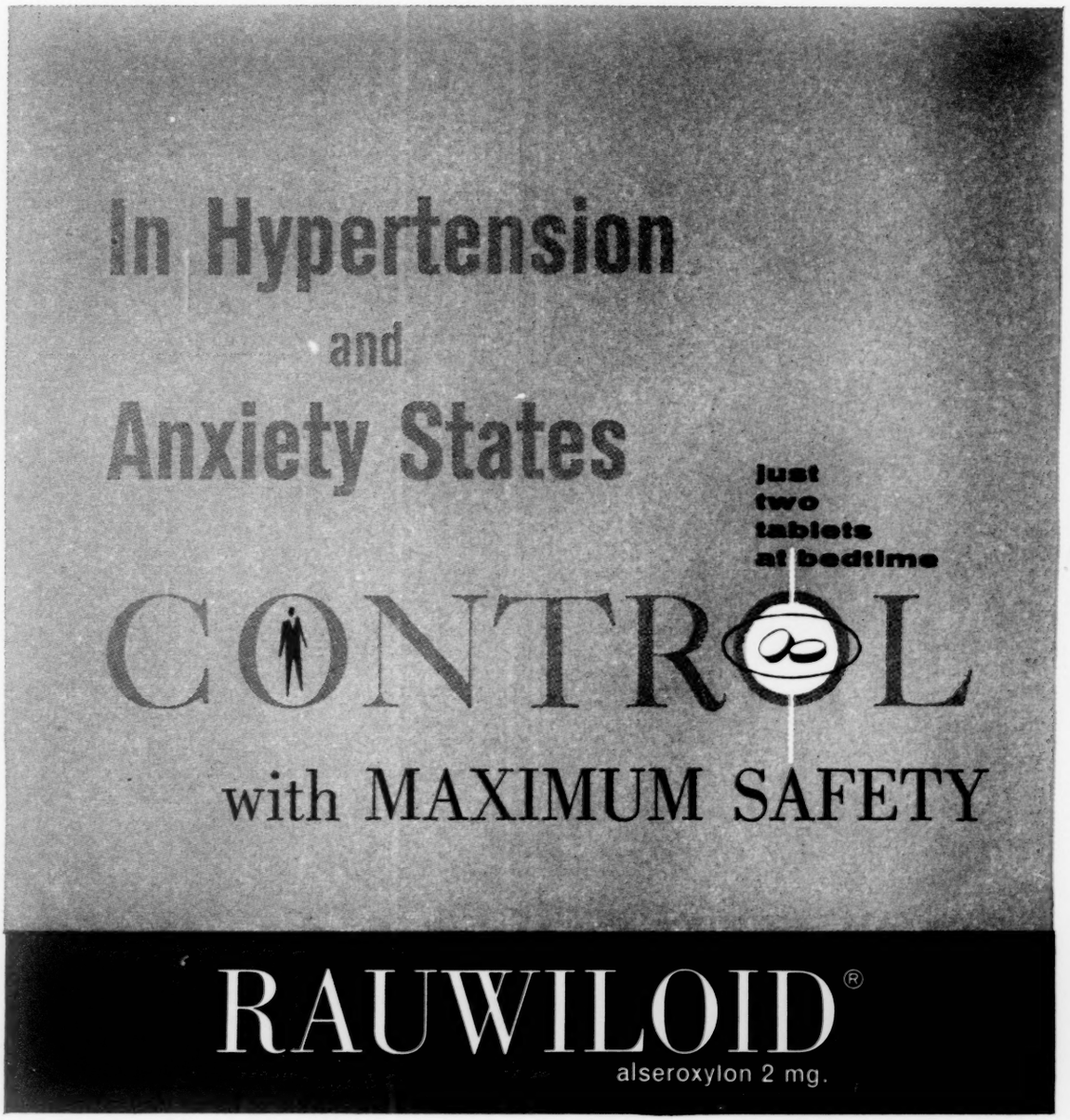
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In Hypertension
Simplicity of control based on negligible incidence of serious side actions, simplicity of dosage, and applicability to a wide range of patients.

In Anxiety States
Rauwiloid is outstanding for its calming, nonsoporific sedation in anxiety states...with or without hypertension.

Compatible with other antihypertensive medications. Potentiates therapeutic action of more potent agents and permits their use in reduced and better tolerated dosage.

When more potent hypotensive action is needed, prescribe one of these convenient single-tablet combinations

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alseroxylon 1 mg. and alkavervir 3 mg.

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Rauwiloid*® + *Hexamethonium
alseroxylon 1 mg. and hexamethonium chloride dihydrate 250 mg.

Patients with severe hypertension often can be maintained on Rauwiloid alone after desired blood pressure levels are reached with combination medication.

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In convenient aerosol spray—for the treatment of dermatoses, particularly in out-of-reach problem areas—a fresh approach to therapy

- the superior anti-inflammatory effect of Kenalog Spray[1-7] provides anti-inflammatory, antiallergic, and antipruritic relief in acute, exudative, weeping lesions, even in extensive, out-of-reach problem areas.
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- metabolic studies show electrolyte disturbance does not occur when Kenalog is applied topically.[1, 4, 5]
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Indications: Atopic dermatitis, contact dermatitis, eczematous dermatitis, neurodermatitis, seborrheic dermatitis, insect bites, pruritus ani and vulvae, lichen simplex chronicus, exfoliative dermatitis, stasis dermatitis, nummular eczema, sunburn.

Dosage: Apply the spray to the affected areas from a distance of 3 to 6 inches, t.i.d. or q.i.d. A 3-second spray (delivering approximately 0.1 mg. of triamcinolone acetonide) covers an area about the size of the hand. *Cover the eyes when using Kenalog Spray on or near the face.*

Supply: Kenalog Spray in 50 and 150 Gm. containers of 3.3 mg. and 10 mg. triamcinolone acetonide respectively. Also available — Kenalog Cream (0.1%), Kenalog Ointment (0.1%) and Kenalog Lotion (0.1%).

References: 1. Reports to the Squibb Institute for Medical Research. 2. Howell, C. M.: Squibb Clin. Res. Notes *1*:5 (Oct.) 1958. 3. Goodman, J. J.: Squibb Clin. Res. Notes *1*:1 (Oct.) 1958. 4. Smith, J. G., Jr.; Zawisza, R. J., and Blank, H.: Squibb Clin. Res. Notes *1*:6 (Oct.) 1958. 5. Fitzpatrick, T. B.; Crowe, F. W., and Walker, S. A.: Squibb Clin. Res. Notes *1*:1 (Oct.) 1958. 6. Lerner, A. B.: Squibb Clin. Res. Notes *1*:2 (Oct.) 1958. 7. Robinson, R. C. V.: Bull. School of Med., U. Maryland 43:54 (July) 1958.

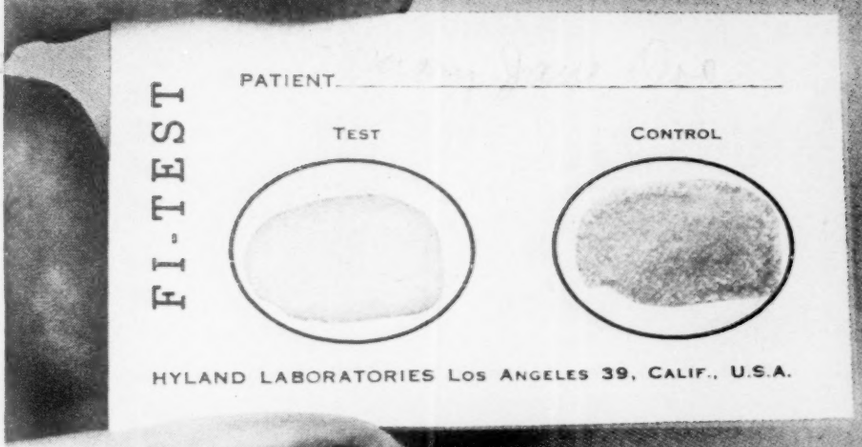
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FI-TEST indicates whether fibrinogen content is above or below 100 mg-%, the concentration considered critical. Easy-to-read results indicate promptly whether or not replacement fibrinogen is needed. (If reading shows a normal fibrinogen level, needless replacement therapy may be avoided and the physician is alerted to seek another explanation for continued bleeding.)

Supplied in compact ready-to-use kits containing complete materials for 6 determinations.



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Fear, agitation, and resistance often hinder medical diagnosis and treatment.

SPARINE alleviates agitation, overcomes resistance, placates fears.

In addition to calming the patient, SPARINE controls other interfering symptoms: nausea, vomiting, and hiccups.

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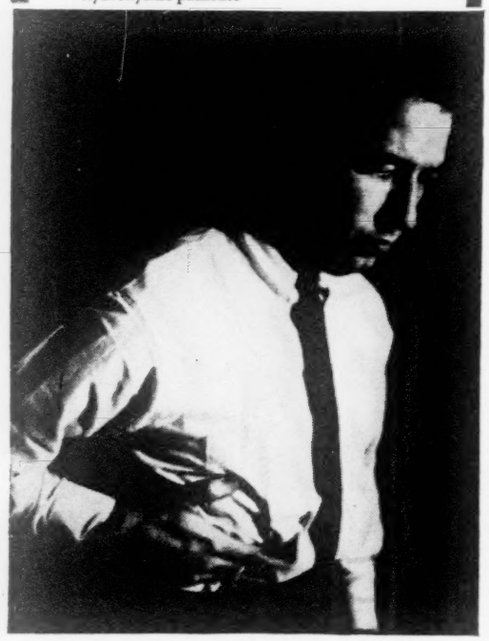
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VISTARIL®

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IN BRIEF

VISTARIL, by allaying anxiety and reducing nervous tension, helps relieve functional pain and discomfort in such conditions as gastroduodenal ulcers, nervous dyspepsia, esophageal spasm and gastroenteritis. In addition, VISTARIL has been reported to lower both the acidity and volume of gastric secretions.

INDICATIONS: VISTARIL is a rapid-acting tranquilizer with a wide margin of safety. It is effective in the symptomatic treatment of a wide variety of neuroses and other emotional disturbances manifested by anxiety, apprehension or fear—whether occurring alone or complicating a physical illness.

ADMINISTRATION & DOSAGE: VISTARIL dosage varies with individual requirements and ranges from 25 mg. t.i.d. to 100 mg. q.i.d. for adults. The usual dosage for children *under* 6 years is 50 mg. daily in divided doses; children *over* 6 years — 50 to 100 mg. in divided doses. For parenteral dosage information consult package insert.

SIDE EFFECTS: Drowsiness may occur in some patients; if so, it is usually transitory, disappearing within a few days of continued therapy or upon reduction of dosage. Dryness of mouth may be encountered at higher doses.

PRECAUTIONS: The potentiating action of hydroxyzine is mild, but must be taken into consideration when the drug is used in conjunction with central nervous system depressants.

SUPPLY: *Capsules* — 25, 50, and 100 mg. *Oral Suspension* — 25 mg. per 5 cc. teaspoonful. *Parenteral Solution* (as the HCl) — 10 cc. vials and 2 cc. Steraject® Cartridges, 25 mg. per cc.; 2 cc. ampules, 50 mg. per cc.

Detailed professional information is available on request from Pfizer Laboratories Medical Department.

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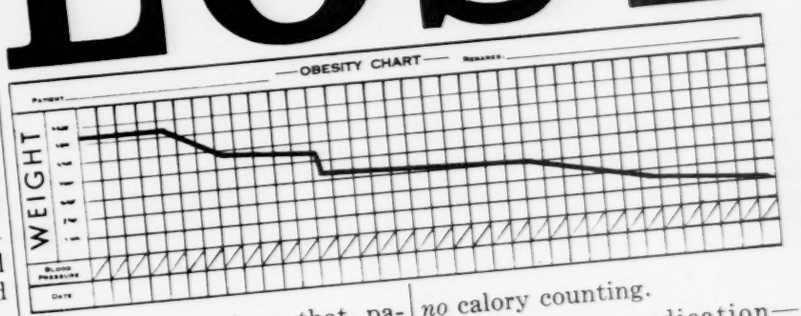
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9,000 TONS
OF EXCESS WEIGHT
LOST



Case histories show that patients lose an average of 6 lbs. per 100 tablets of Obedrin—making a total weight loss of 18,000,000 lbs. since Obedrin was introduced.

Obedrin and the 60-10-70 Basic Plan succeed where other reducing regimens fail. The Obedrin Basic Plan provides three essentials for weight reduction:

1. Supervision by the physician—Patients need the emotional support and discipline that only the physician can contribute.

2. A balanced eating plan—The 60-10-70 Basic Plan offers the patient a wide variety of menus, carefully calculated to supply a minimum of 60 grams of carbohydrate, 10 grams of fat, and 70 grams of protein each day, with *no* calory counting.

3. Supportive medication—Each capsule or tablet of Obedrin provides:

• Semoxydrine® HCl (methamphetamine HCl), 5 mg., for its anorexigenic and mood-lifting effects

• Pentobarbital, 20 mg., to guard against excitation

• Thiamine Mononitrate, 0.5 mg., Riboflavin, 1 mg., and Nicotinic Acid (Niacin), 5 mg., to supplement the diet

• Ascorbic Acid, 100 mg., to help mobilize tissue fluids

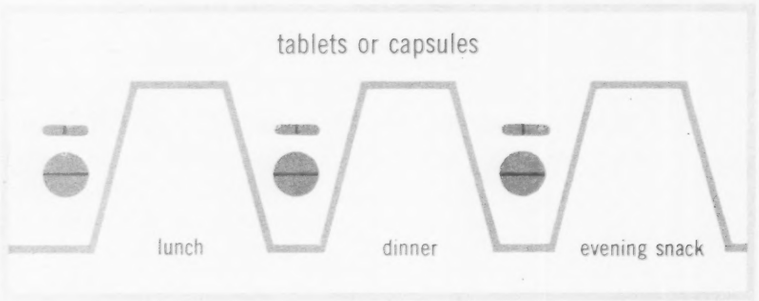
Obedrin and the 60-10-70 Basic Plan help the overweight patient establish correct eating habits...first, to lose excess pounds and then, more important, to *maintain* optimum weight.

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A FLEXIBLE DOSAGE FORM PROVIDES DEPENDABLE CONTROL OF APPETITE



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A dependable anorexigenic agent
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Write for 60-10-70 Basic Plan menus and weight charts.

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potentiated therapy for mild to moderate hypertension

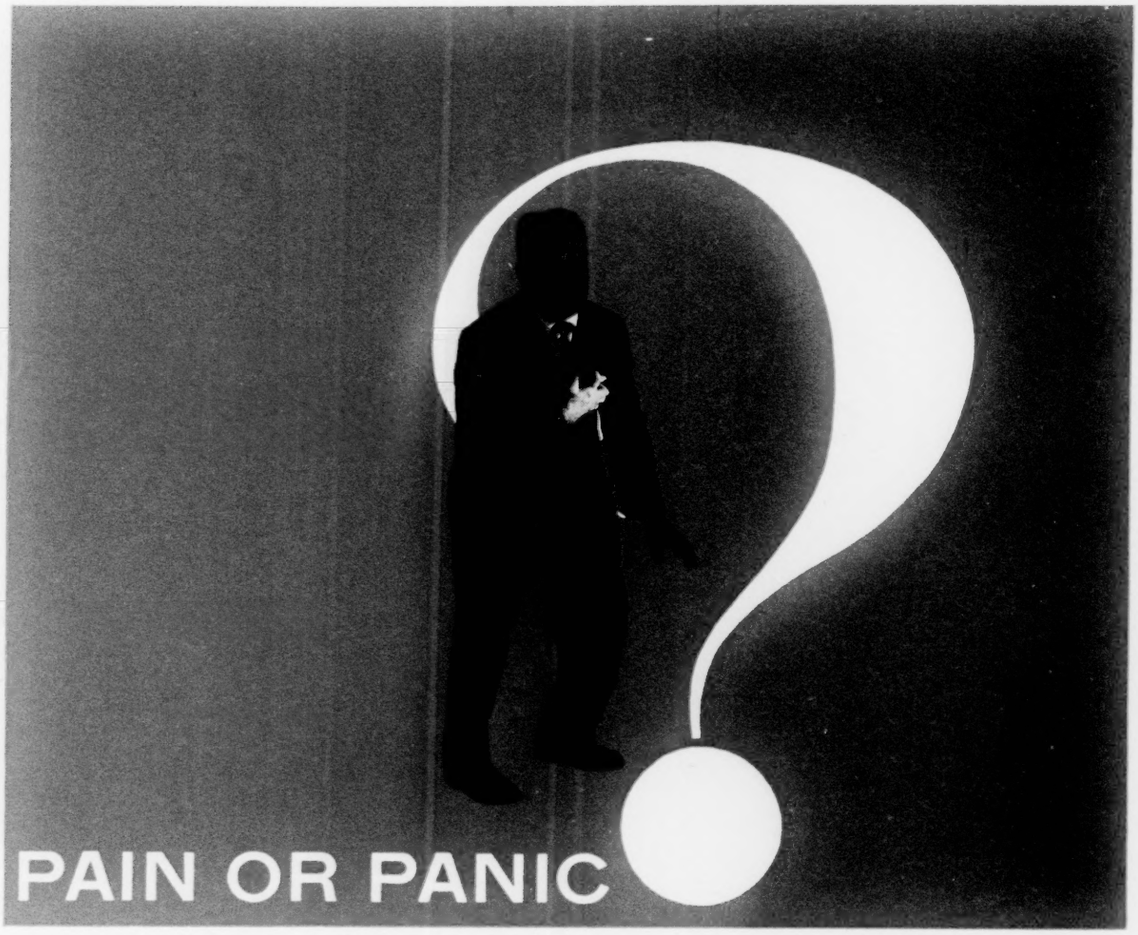
Consider the benefits of Singoserp-Esidrix if it's mild to moderate hypertension (especially if edema is a complicating symptom). Singoserp, a man-made analog of reserpine, lowers blood pressure but seems to cause fewer side effects than natural rauwolfia compounds. When Singoserp is potentiated by Esidrix, blood pressure is lowered more effectively than with single-drug therapy. SUPPLIED IN TWO STRENGTHS: Singoserp-Esidrix *Tablets #2* (each containing 1 mg. Singoserp and 25 mg. Esidrix) and Singoserp-Esidrix *Tablets #1* (each containing 0.5 mg. Singoserp and 25 mg. Esidrix). Complete information available on request.



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(syrosingopine and hydrochlorothiazide CIBA)

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Your angina patient hardly knows where one leaves off and the other begins. What he does recognize is the relief he gets from **both** when you prescribe CARTRAX.

FOR ANGINAL PAIN

PETN (pentaerythritol tetranitrate) is "... the most effective drug currently available for prolonged prophylactic treatment of angina pectoris."[1]

FOR CARDIAC PANIC

"I favor ATARAX [as the tranquilizer for the anxious cardiac] ... because there is an absence of side effects with this drug, and also because in cardiacs who are troubled with ectopic beats, ATARAX has a quinidine-like action."[2]

PETN + ATARAX®*

TOGETHER ONLY IN

CARTRAX®

Dosage: Begin with 1 to 2 yellow CARTRAX "10" tablets (10 mg. PETN plus 10 mg. ATARAX) 3 to 4 times daily. When indicated, this may be increased by switching to pink CARTRAX "20" tablets (20 mg. PETN plus 10 mg. ATARAX). For convenience, write "CARTRAX 10" or "CARTRAX 20." **Supplied:** In bottles of 100. Prescription only.

References: 1. Russek, H. I.: Postgrad. Med. 19:562 (June) 1956. 2. Russek, H. I.: Presented at the Symposium on the Management of Cardiovascular Problems of the Aged, Dade County Medical Association, Miami Beach, April 12, 1958.

*brand of hydroxyzine

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BELLERGAL SPACETABS — Bellafoline 0.2 mg., ergotamine tartrate 0.6 mg., phenobarbital 40.0 mg.
Dosage: 1 in the morning, and 1 in the evening.
BELLERGAL TABLETS — Bellafoline 0.1 mg., ergotamine tartrate 0.3 mg., phenobarbital 20.0 mg.
Dosage: 3 to 4 daily. In more resistant cases, dosage begins with 6 tablets daily and is slowly reduced.

SANDOZ

in premenstrual tension



clinicians report rapid relief with

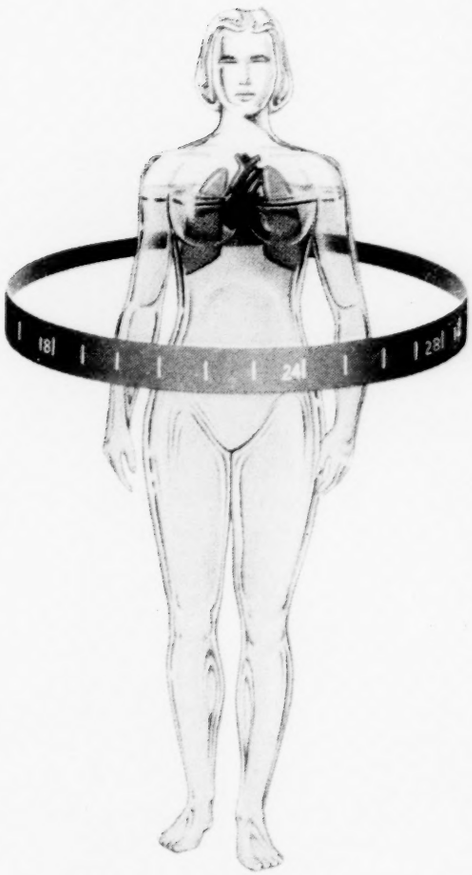
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increased potency–without corresponding increase in side effects

Fuchs, M. and Moyer, J.:
Diseases of the Chest 35:314, (March) 1959.

"Premenstrual edema is present in 40% of women and...consists of weight gain, subcutaneous edema, emotional lability, breast turgidity, anxiety and tension." In addition to controlling the objective symptoms of premenstrual tension, HYDRODIURIL may afford relief of subjective complaints including tension, nervousness and headache.



DOSAGE: 25 to 50 mg. of HYDRODIURIL once or twice a day, beginning the first morning of symptoms and continuing until the onset of the menses.

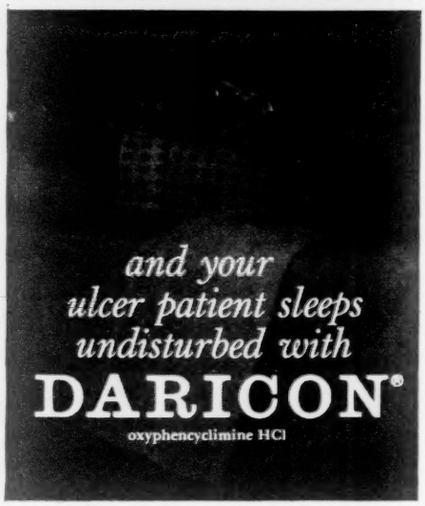
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HYDRODIURIL is a trademark of Merck & Co., INC.

Additional information on HYDRODIURIL is available to the physician on request.

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IN BRIEF

Just 2 DARICON tablets — one in the morning and one at bedtime — can provide round-the-clock relief from ulcer pain.

INDICATIONS: DARICON is an effective adjunct in the management of duodenal ulcers and other gastrointestinal disorders caused by spasm, hypermotility or hypersecretion.

ADMINISTRATION & DOSAGE: One 10 mg. tablet b.i.d. (morning and night). Adjust according to response.

SIDE EFFECTS: As with other anticholinergic agents, undesirable effects occasionally appear. Dryness of mouth is the most common of these peripheral effects. Blurring of vision, constipation, and urinary hesitancy are rare, and usually can be minimized with dosage adjustment or may disappear on continued therapy.

PRECAUTIONS: Care should be exercised in using DARICON in patients with prostatic cancer, in whom urinary retention may occur. In patients with glaucoma, DARICON should be used only with ophthalmologic supervision and approval.

SUPPLY: White, scored 10 mg. tablets.

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and related disorders such as

indigestion • heartburn • spastic colon

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OXAINE*

containing a gastric mucosal anesthetic

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INDICATIONS

Gastritis: In chronic gastritis, OXAINE provides relief of substernal pain and upper abdominal distress and permits patients a pronounced increase in the volume and variety of food intake. Medical management of gastritis should include physical rest, small meals of bland foods, and adequate fluid intake. In acute phases, fasting, bed rest, and gastric suction may be indicated. Initial feeding should consist of clear liquids rather than the fatty foods often prescribed for peptic-ulcer patients.

Gastritis occurs more frequently than peptic ulcer; both may occur concurrently, however. The pain pattern of chronic gastritis is usually the reverse of that typical of peptic ulcer. Food usually provides relief of ulcer; the distress of gastritis occurs on ingestion of food. Initially, the pain is intermittent; later, it may become constant and intractable. One of the earliest symptoms is upper abdominal pain following the major meal. Eventually, the same pain recurs after almost *every* meal; then belching after the major meal becomes distressing. The upper abdominal discomfort, belching, and restlessness increase in frequency and severity. Then nausea, vomiting, and general weakness become complicating factors. Constipation, prolonged fluid deficit and intolerance of fresh fruits and vegetables may be associated. Fear of cancer may be disabling. Physical examination often shows only tenderness on light percussion or palpation of the upper abdomen. Abdominal distention may be noticeable. Gastric analysis may show hyper- or hypo-chlorhydria.

Esophagitis: OXAINE therapy relieves discomfort and lessens the frequency with which esophageal dilatation is required. Patients should sleep with the back and head elevated to minimize reflux of gastric contents into the lower end of the esophagus.

Irritable colon syndrome: In patients with this common functional disorder of the gastrointestinal tract, OXAINE diminishes the sensitivity of the gastrocolic reflex. Among a variety of vague complaints are discomfort in the left lower abdomen and sometimes crampy pain and nausea. The urge to defecate often follows ingestion of food. A low residue, bland diet is often indicated; also attention to psychosomatic aspects.

DOSAGE AND ADMINISTRATION. The recommended adult dose is one or two teaspoonfuls four times daily, 15 minutes before meals and at bedtime. Do not exceed recommended dosage.

PRECAUTIONS. Adequate diagnostic studies are recommended. The possibility of gastrointestinal carcinoma should be considered in patients with protracted or recurrent indigestion. Constipation, which may be aggravated by therapeutic doses of OXAINE, should be prevented by adequate fluid intake and dietary roughage or mineral oil preparation (such as PETROGALAR®).

If the dose of OXAINE exceeds 12 teaspoonfuls a day, some patients may experience dizziness, faintness, or drowsiness. Two cases of glossitis of the hypersensitivity type have been reported in extensive clinical studies. One observed case of skin eruption was relieved by discontinuation of medication.

SAFETY: A wide margin of safety exists between the effective dose and oral toxic dose of oxethazaine in aluminum hydroxide gel. In mice, the oral LD_{50} of oxethazaine when suspended in aluminum hydroxide gel is 1012 mg. of base per kg. The effective dose of OXAINE, one or two teaspoonfuls, contains 10 or 20 mg. of oxethazaine respectively; this is only 0.2 or 0.4 mg. per kg. of body weight for a 50 kg. human being. In human beings, toxic effects have not been observed on continued ingestion of recommended therapeutic doses.

For further information on prescribing and administering OXAINE ***see descriptive literature, available on request.***

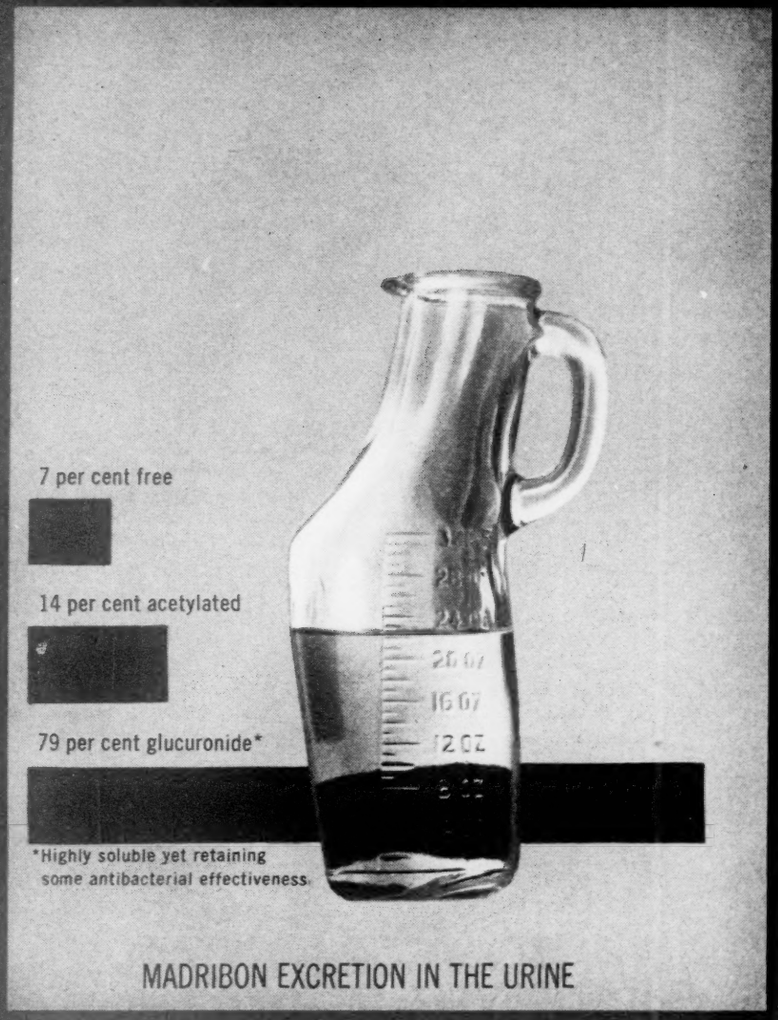
†Deutsch, E., and Christian, H.J.: Chronic gastritis; histological criteria for management and medical treatment with a mucosal anesthetic in aluminum hydroxide, J. Am. Med. Assoc. *169*:2012 (April 25) 1959.

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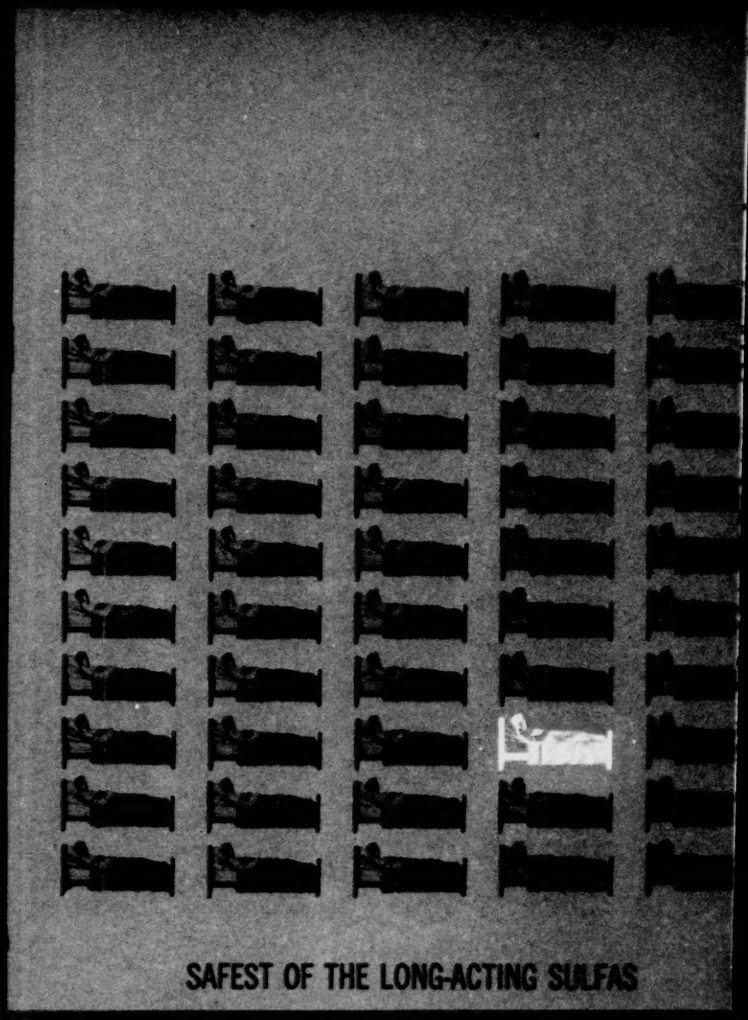
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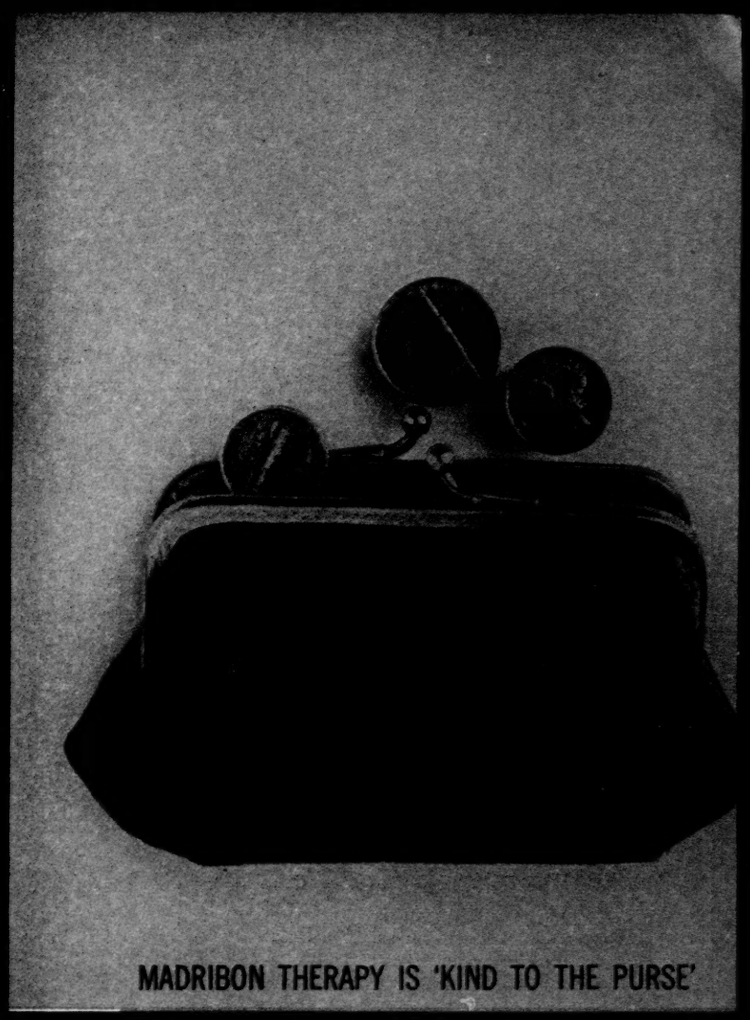
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Chausse, P. Gibaud and J. Bottin, *J. méd. Bordeaux, 136*:713, 1959. **35.** B. H. Leming, Jr. and C. Flanigan, Jr., Scientific Exhibit, Annual Meeting of the American Medical Association, Atlantic City, N. J., June 1959. **36.** J. C. Elia, *ibid.* **37.** M. J. Mosely, Jr., *J. Nat. M.A., 51*:258, 1959. **38.** H. Schoenfeld and W. Sommerfeld, *Aerztl. Wchnschr., 14*:619, 1959. **39.** H. Ptasnik, *Medizinische,* (31/32), 1437, 1959. **40.** P. Rentchnick and J. Lagier, *Schweiz. med. Wchnschr., 89*:894, 1959. **41.** R. E. Bagdon, L. O. Randall and W. A. Leff, *Ann. New York Acad. Sc., 82*:(Art. 1), 3, 1959. **42.** W. F. DeLorenzo and R. J. Schnitzer, *ibid.*, p. 10. **43.** W. P. Boger and J. J. Gavin, *ibid.*, p. 18. **44.** B. H. Leming, Jr. and C. Flanigan, Jr., *ibid.*, p. 31. **45.** T. D. Michael, *ibid.*, p. 40. **46.** S. M. Finegold, Z. Kudinoff, H. O. Kendall and V. E. Kvinge, *ibid.*, p. 44. **47.** W. J. Grace, *ibid.*, p. 51. **48.** J. C. Elia, *ibid.*, p. 52. **49.** L. E. Skinner, *ibid.*, p. 57. **50.** G. A. Moore, *ibid.*, p. 61. **51.** C. W. Daeschner, *ibid.*, p. 64. **52.** E. H. Townsend, Jr. and A. Borgstedt, *ibid.*, p. 71. **53.** S. Krugman, Discussant, *ibid.*, p. 78. **54.** S. W. Levy, *ibid.*, p. 80. **55.** M. M. Cahn and E. J. Levy, *ibid.*, p. 84. **56.** M. Sierp and J. W. Draper, *ibid.*, p. 92. **57.** W. S. Kiser, O. C. Beyer and J. D. Young, *ibid.*, p. 105. **58.** G. Carroll, Discussant, *ibid.*, p. 110. **59.** H. L. Rosenthal and L. Jud, *J. Lab. & Clin. Med., 54*:461, 1959. **60.** A. E. Thill, *Pennsylvania M.J., 62*:1534, 1959. **61.** Council on Drugs, New and Nonofficial Drugs, *J.A.M.A., 171*:1691, 1959. **62.** T. Sakuma, C. W. Daeschner and E. M. Yow, *Am. J.M. Sc., 239*:92, 1960. **63.** J. W. Faulkner and A. F. Morrison, *J. Urol., 83*:181, 1960. **64.** H. Lieb, *Curr. Therap. Res., 2*:66, 1960. **65.** G. D. La Veck, F. de la Cruz and J. Kirschvink, *Antibiotic Med. & Clin. Therapy, 7*:119, 1960. **66.** R. J. Williams, *Antibiotic Med. & Clin. Therapy,* in press. **67.** J. C. Elia, *Mil. Med., 125*:258, 1960. **68.** N. Mulla, *Obst. & Gynec.,* to be published. **69.** B. Pinck, *J. Urol.,* to be published. **70.** J. C. Elia, *Eye Ear Nose & Throat Month.,* to be published. **71.** H. B. Barner, *Antibiotic Med. & Clin. Therapy,* to be published. **72.** J. P. Cappuccio and E. C. Dobbs, *J. Oral Surg.,* to be published. **73.** A. Lattimer, A. J. Simon and M. H. Lepper, *Am. J. M. Sc.,* to be published. **74.** J. C. Elia, *J. Internat. Coll. Surgeons, 33*:446, 1960.

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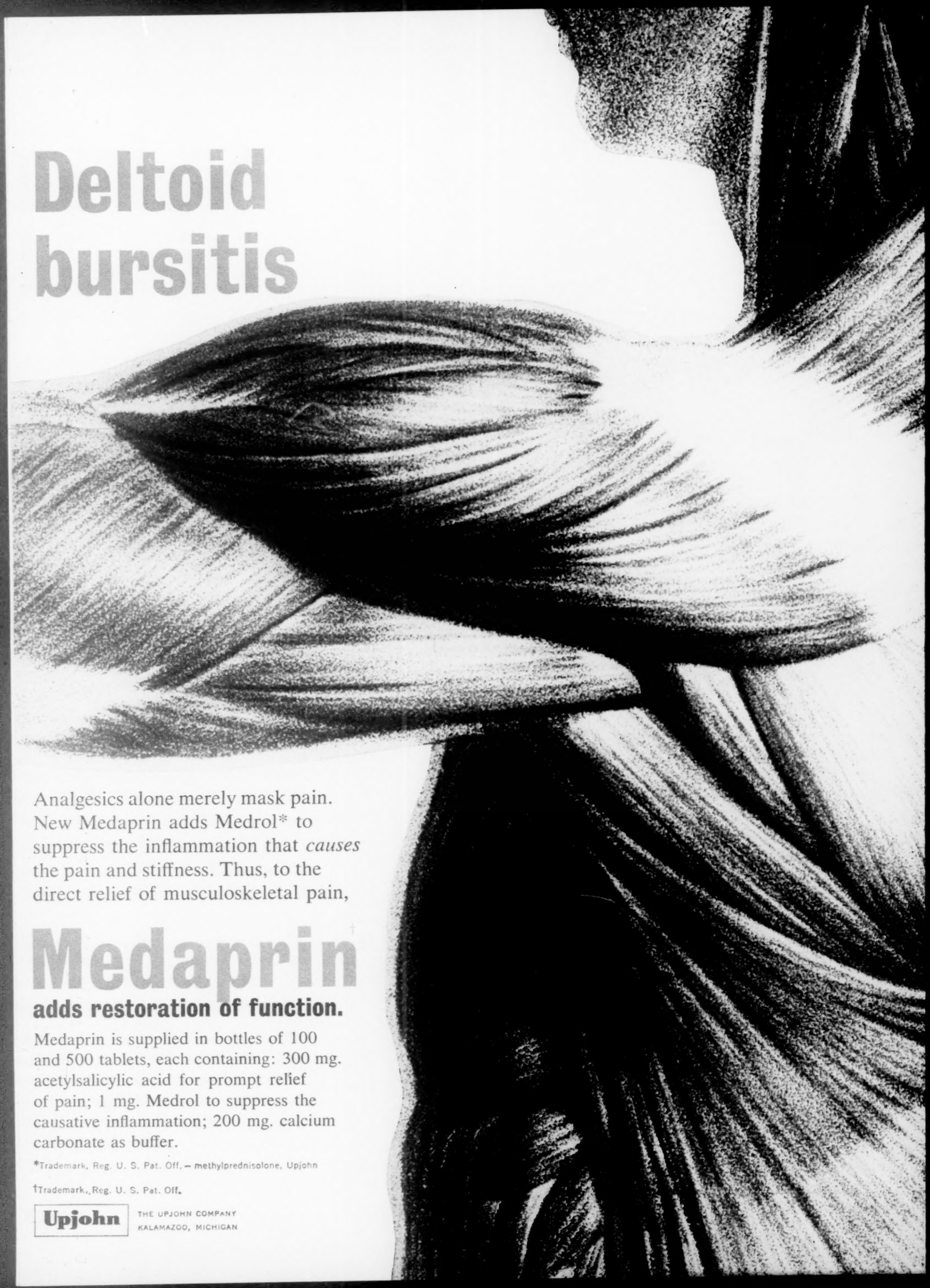
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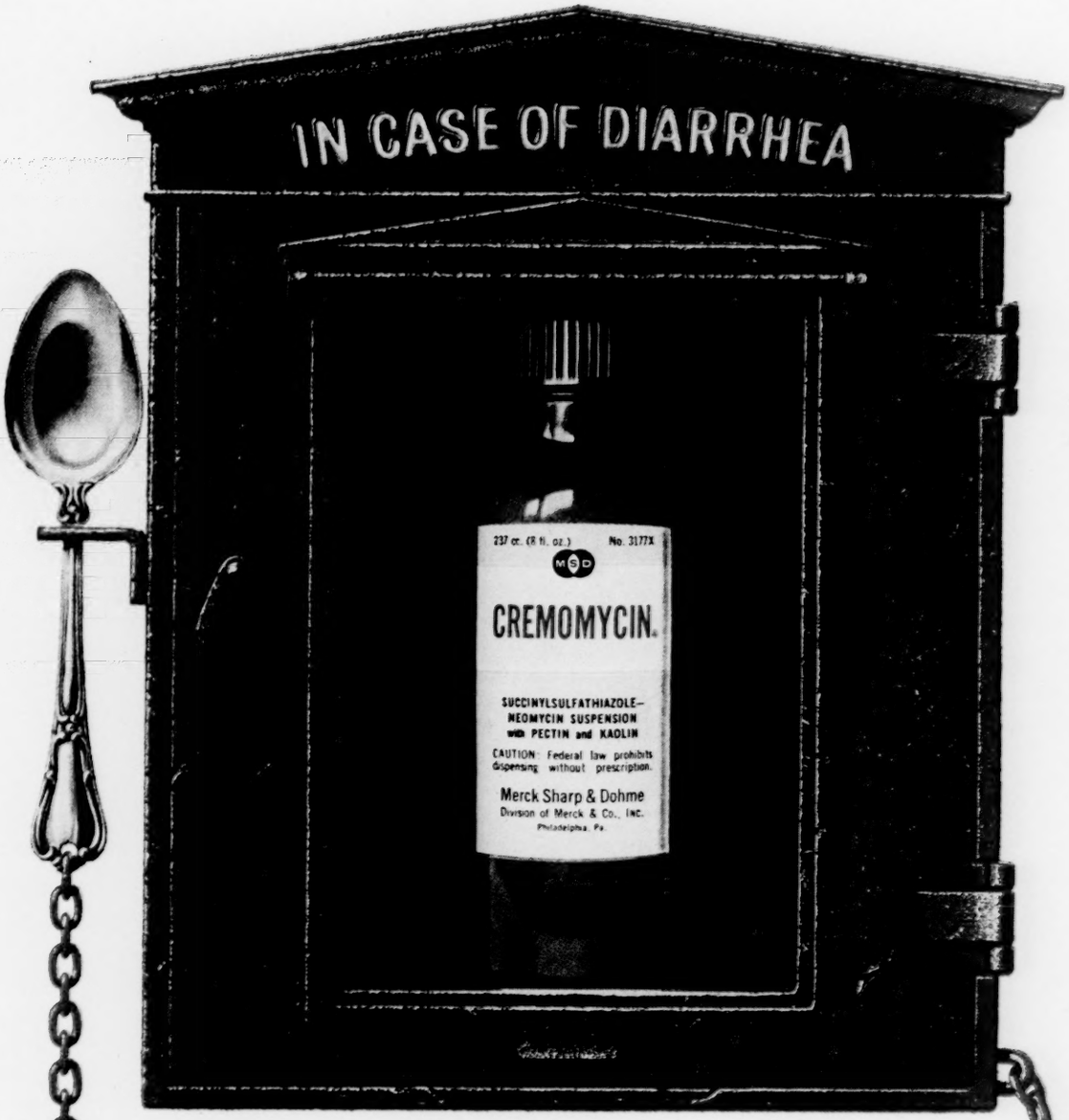
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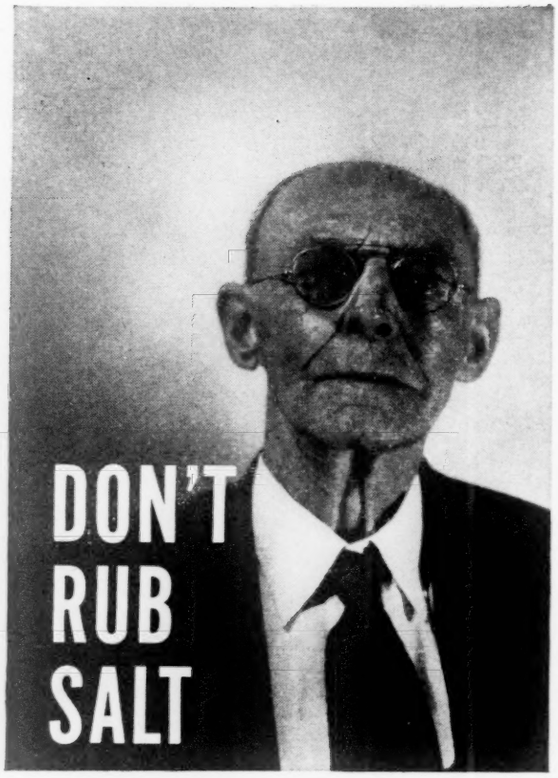
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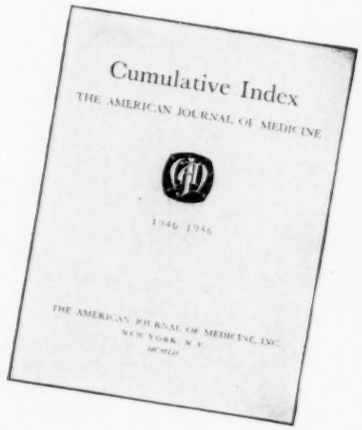
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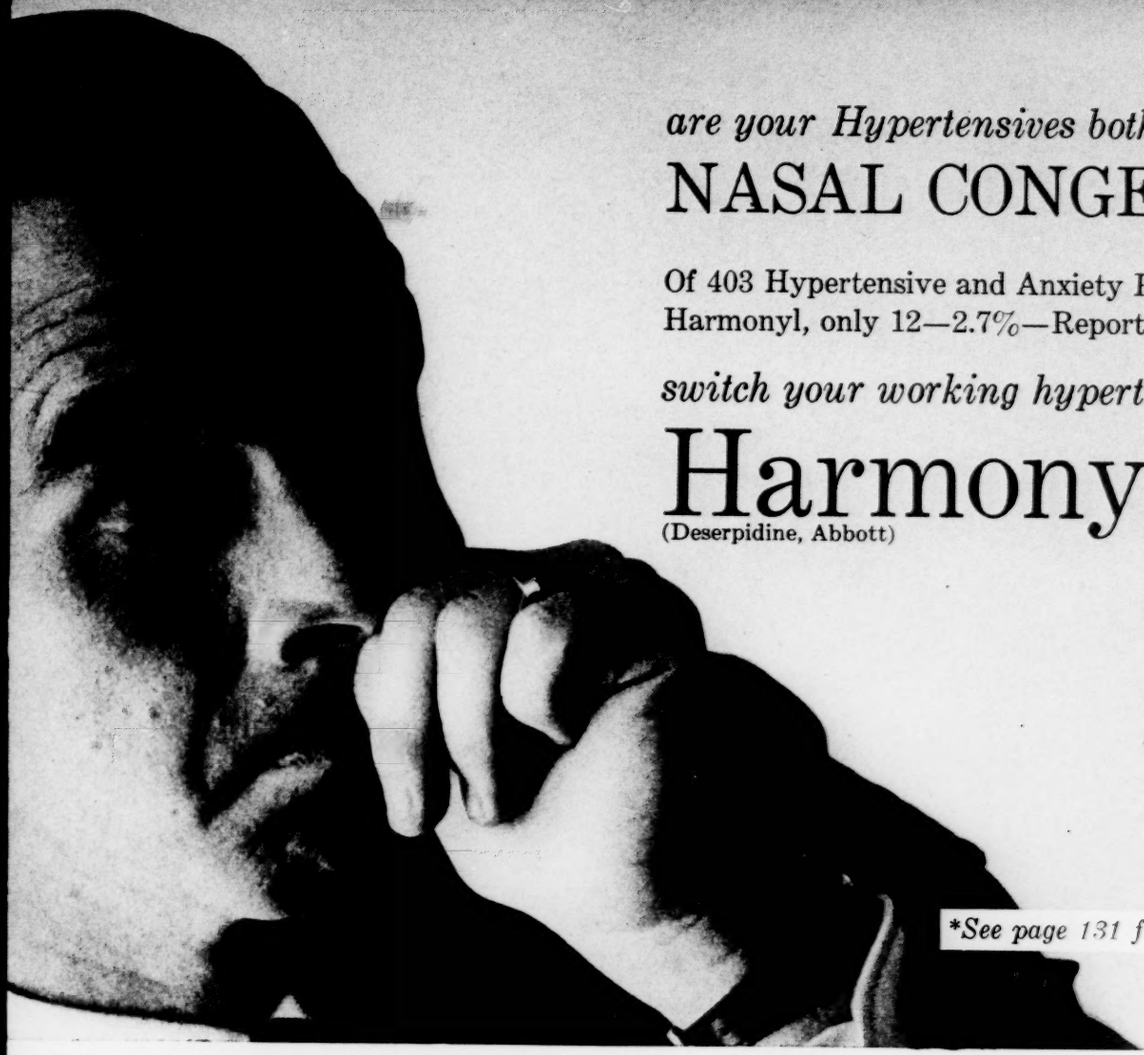
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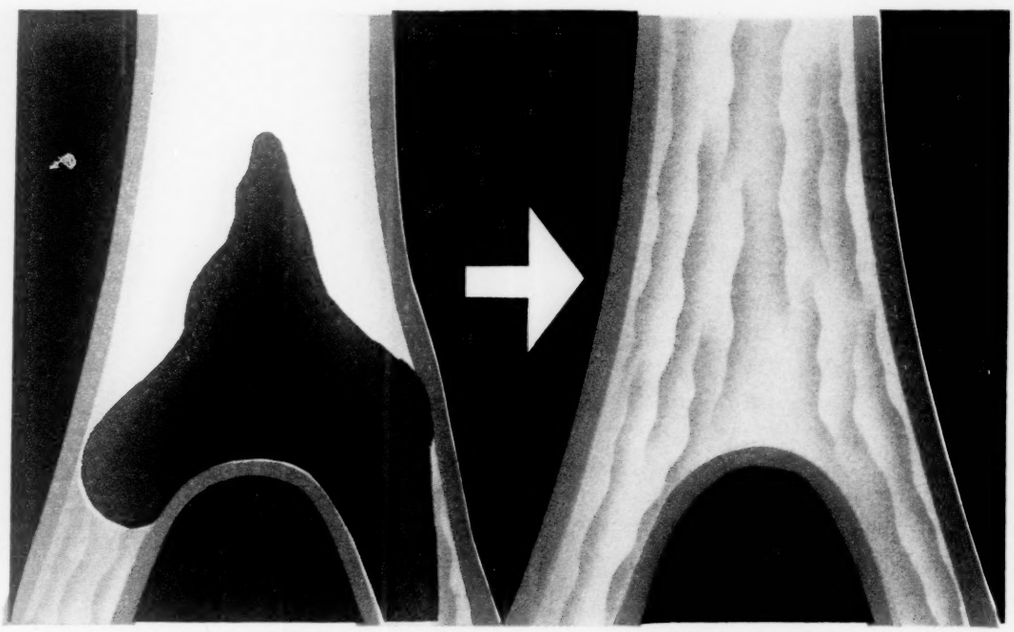
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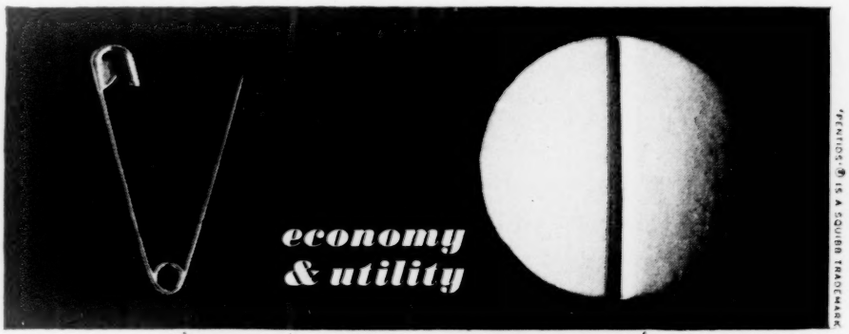
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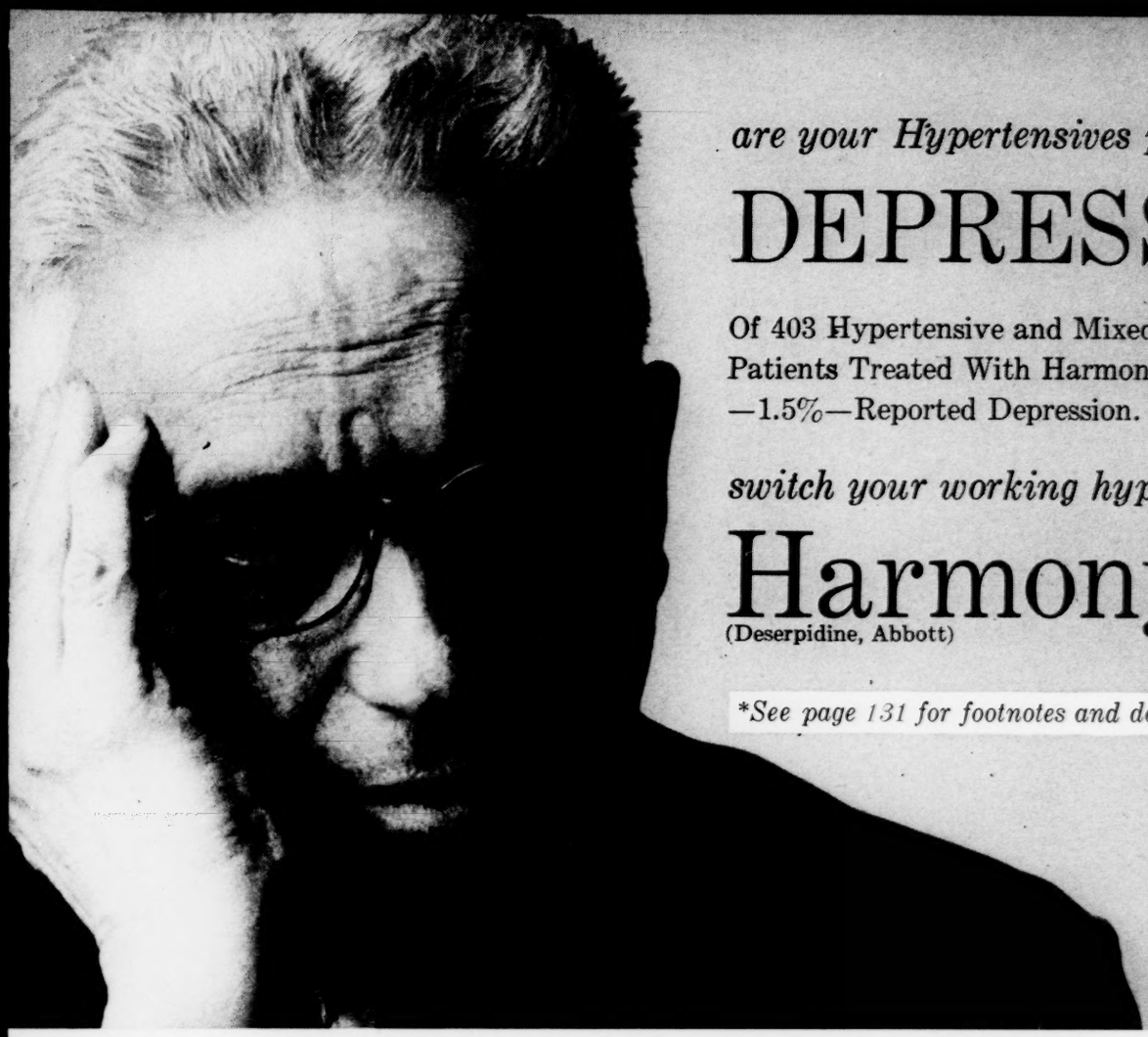
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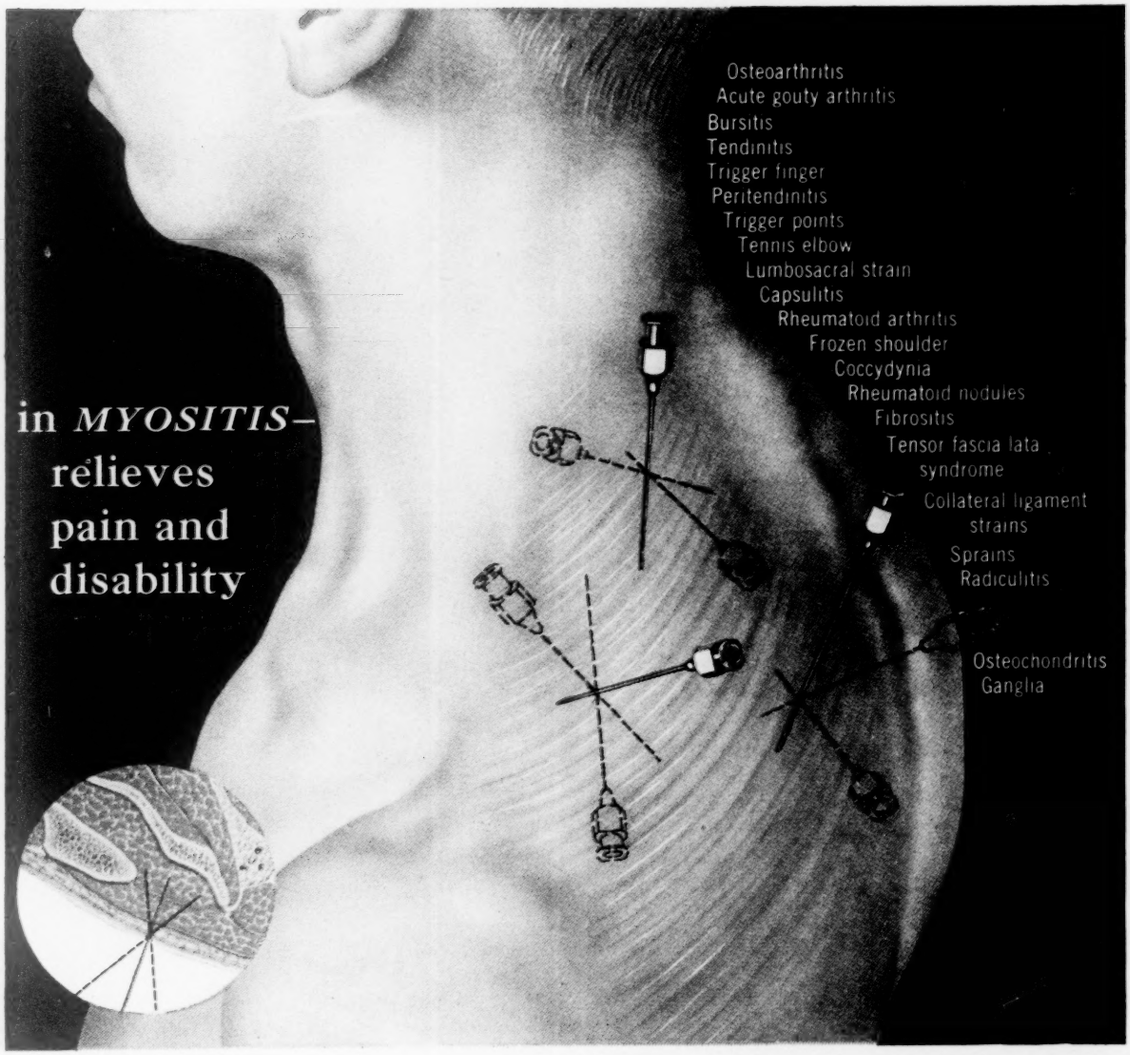
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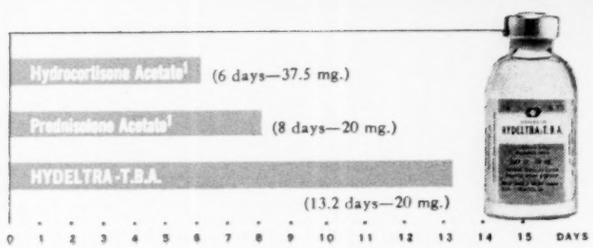
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Of 403 Hypertensive and Mixed Anxiety Patients Treated With Harmonyl, Only 6—1.5%—Reported Depression (mild)

Presented are notes from three clinical studies. Harmonyl was used as sole agent of therapy by 403 mixed hypertensives, and patients with mild functional/psychiatric disorders. Only six reported mild depression concurrent with therapy—an incidence of 1.5%.

Study #1. Two hundred and eighty-three patients treated in private practice, at hospital outpatient clinics, and in office psychiatric practice. Average initial dosage of Harmonyl was 0.1 mg. to 0.25 mg. three or four times daily. No patient received more than 6 mg. daily. Six reports of mild depression.[1]

Study #2. Eighty patients with benign essential hypertension were studied via "double-blind" method. Mean daily dosage of Harmonyl was 0.25 mg., with a minimum dosage of 0.1 mg. and a maximum of 0.5 mg. daily. No reports of depression.[2]

Study #3. Forty patients with hypertension and anxiety neurosis . . . from mild to moderately severe. Half of the group had received other antihypertensive agents at one time or another; these were discontinued before and throughout the study. Usual dosage of Harmonyl was 0.1 mg. three times daily after meals. Sometimes an additional dose was taken at bedtime. No reports of depression associated with this therapy.[3]

Other investigators have found so little indication of depression with Harmonyl therapy that they do not record it as a significant side-effect.

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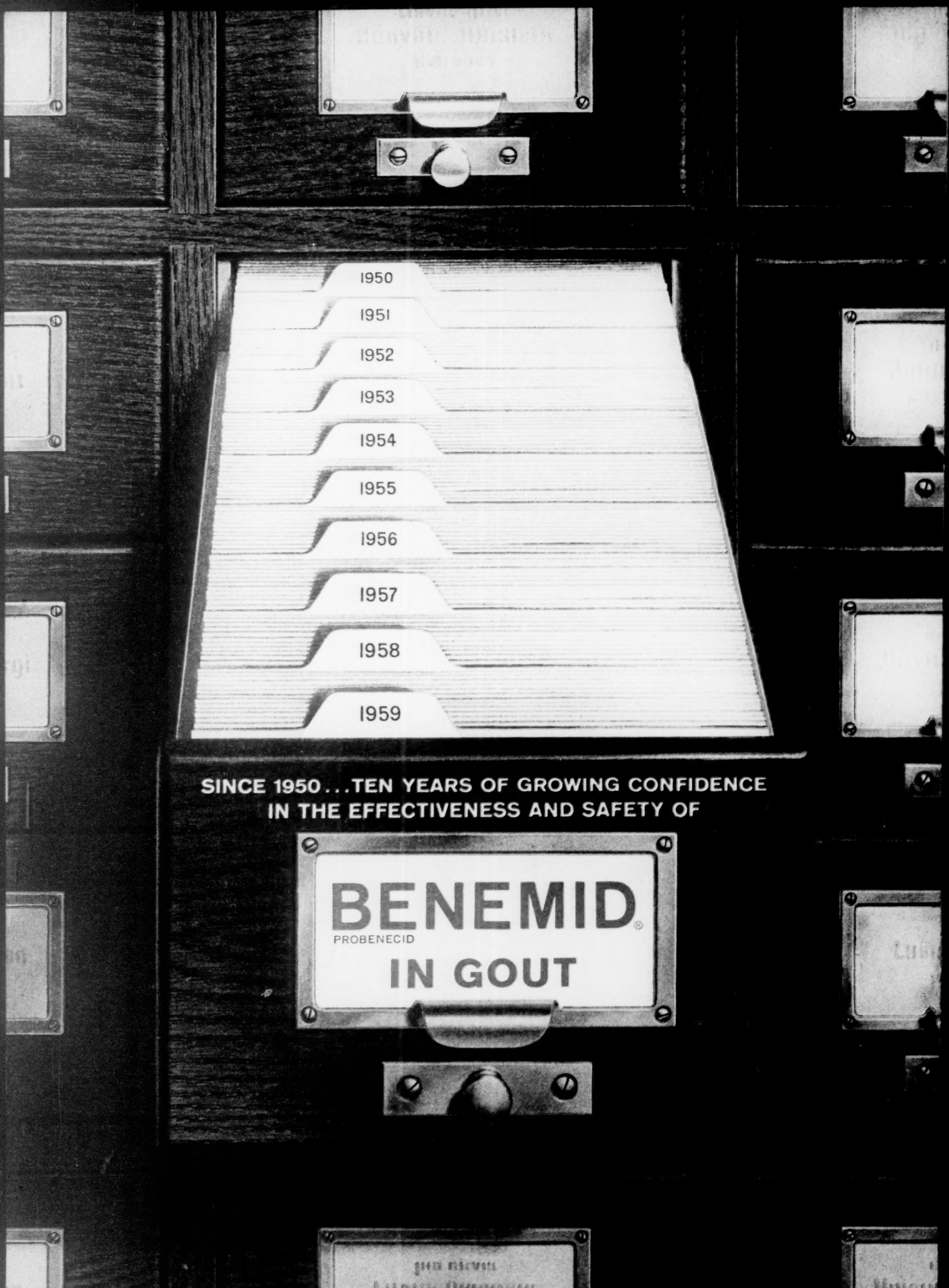


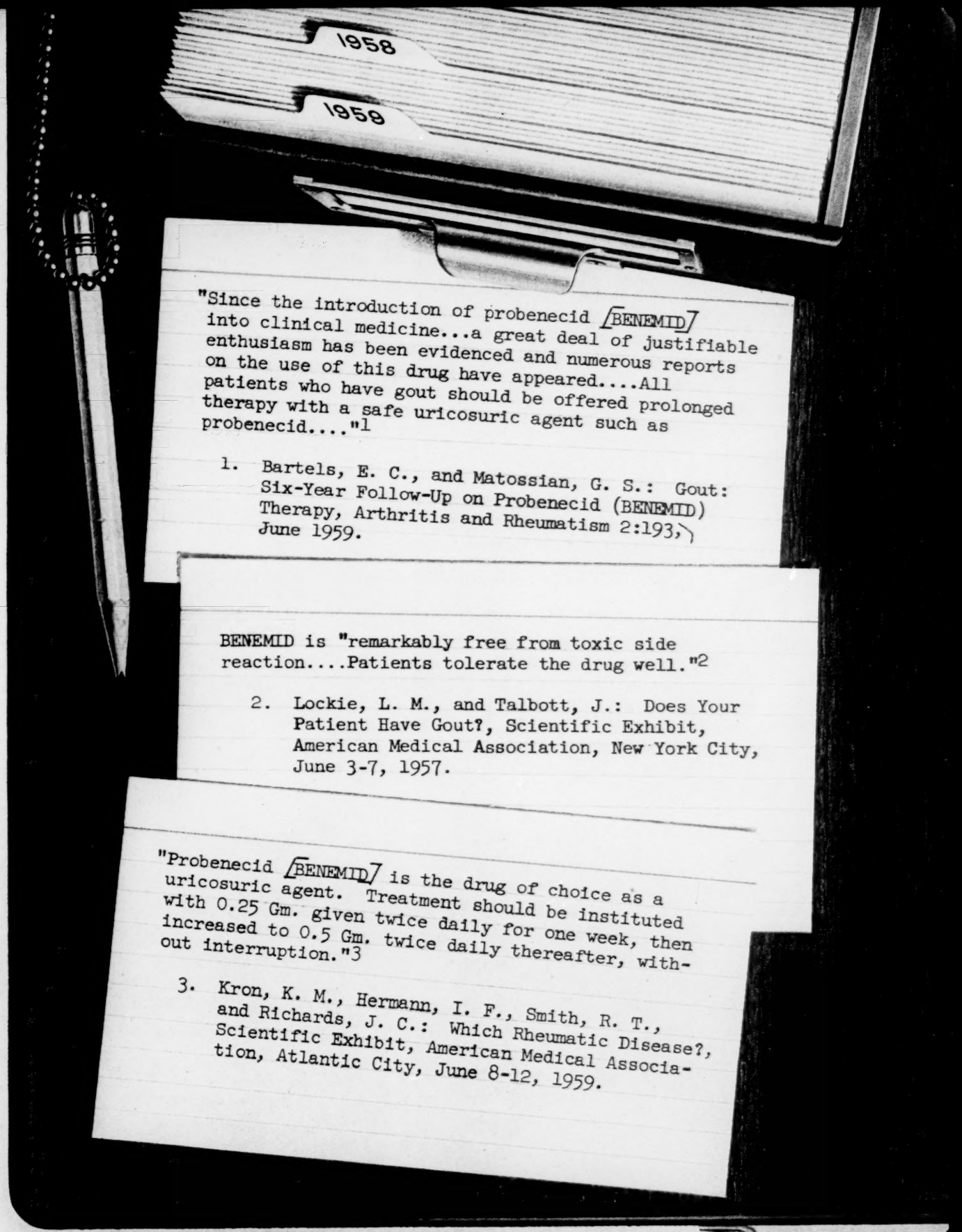
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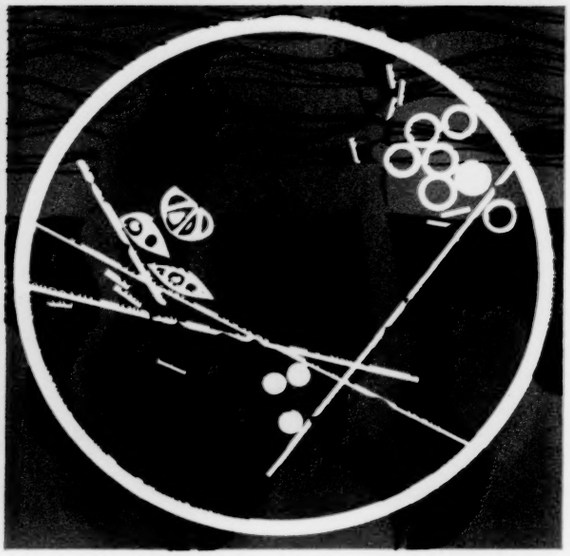
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(1) Fox, H. H.: Antibiot. Med. & Clin. Therapy *6*:85, 1959.
(2) Lubowe, I. I.: Antibiot. Med. & Clin. Therapy *4*:81, 1957.
(3) Murphy, J. C.: Rocky Mountain M. J. *55*:53 (June) 1958.
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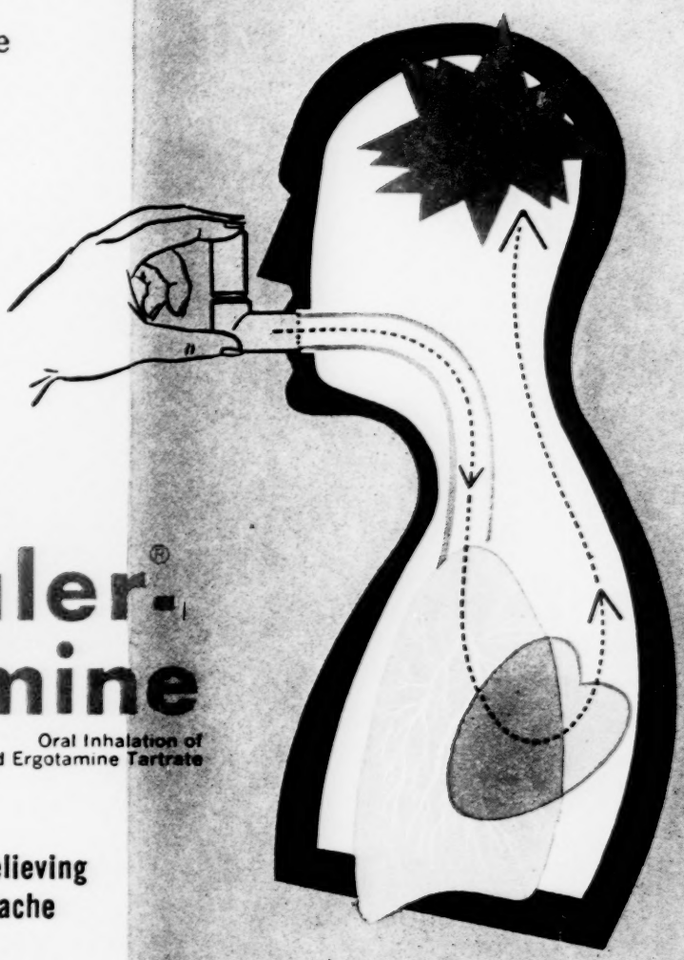
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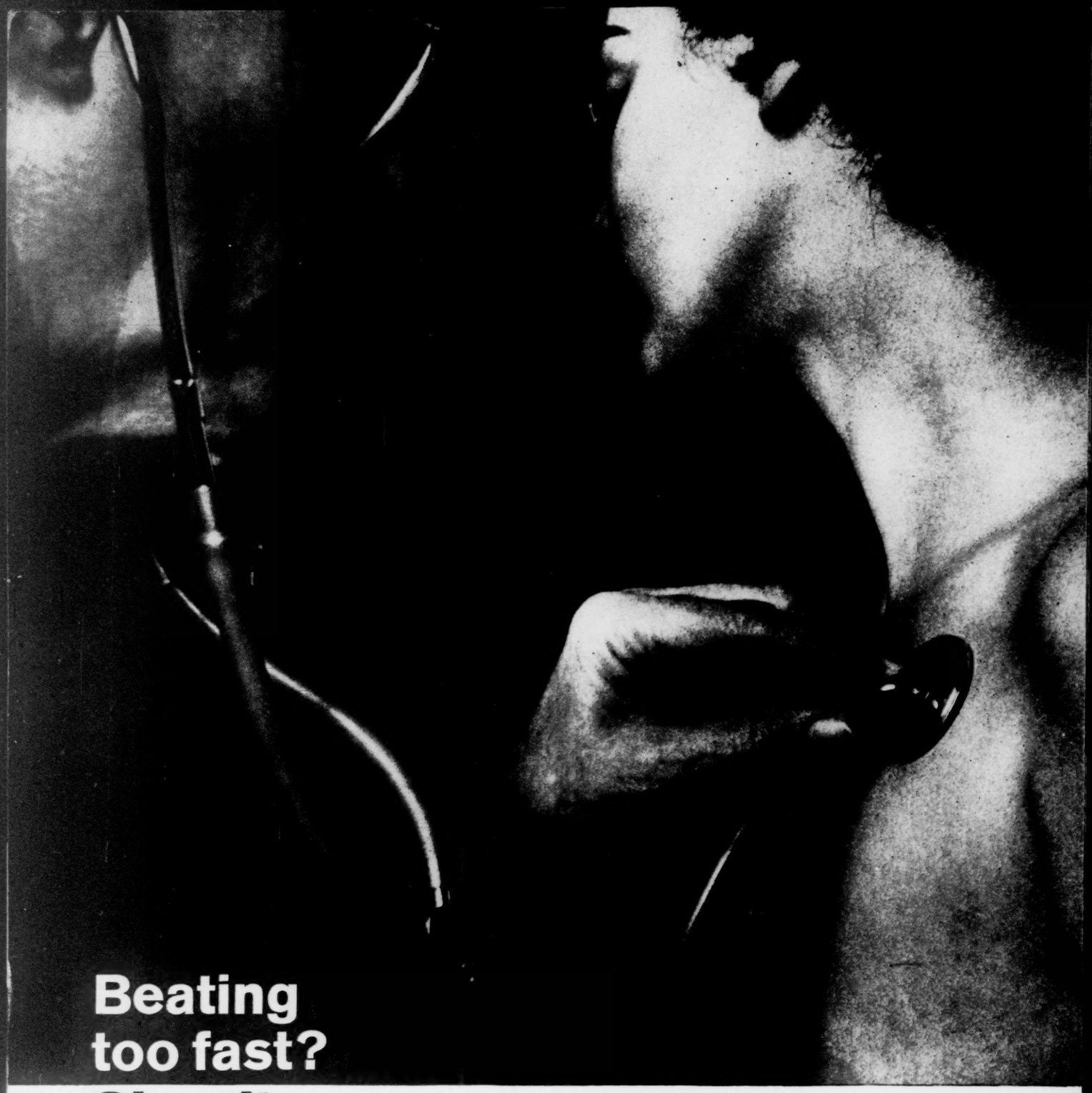
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